The Role of Frontal Cortex in the Generation of Saccadic Eye Movements and Fixation

by

Marc A. Sommer

M.S. Electrical Engineering
Stanford University, 1990

B.S. Biological Sciences and Electrical Engineering
Stanford University, 1989

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SUBMITTED TO THE DEPARTMENT OF BRAIN AND COGNITIVE SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY IN SYSTEMS NEUROSCIENCE
AT THE
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

SEPTEMBER 1995

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Signature of Author _____
Department of Brain and Cognitive Sciences
July 27, 1995

Certified by _____
Peter H. Schiller
Dorothy W. Poitras Professor
Thesis Supervisor

Accepted by _____
Emilio Bizzi
Eugene McDermott Professor in the Brain
and Cognitive Sciences and Human Behavior
Head, Department of Brain and Cognitive Sciences

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Submitted to the Department of Brain and Cognitive Sciences on July 27, 1995 in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Systems Neuroscience

ABSTRACT

Two distinct regions of the rhesus monkey frontal cortex are involved in the generation of saccadic eye movements and fixations: the frontal eye field and the dorsomedial frontal cortex. Previous results have not resolved the relative functions of these two areas. We reversibly inactivated a region of frontal eye field or of dorsomedial frontal cortex with lidocaine. Injections of muscimol or saline served as controls. Inactivation of a frontal eye field severely disrupted the monkey's ability to make contraversive saccades to remembered target locations and to brief target flashes. Frontal eye field inactivation also impaired contralateral fixation. Inactivation of dorsomedial frontal cortex did not affect simple saccades or fixations, but it did cause a deficit in the ability to perform sequences of saccades and fixations.

These results suggest that the two oculomotor regions of frontal cortex have different functions. The frontal eye field may be specialized for generating fixations and saccades to singly presented visual stimuli, especially when their locations must be remembered. The dorsomedial frontal cortex appears to be necessary for coordinating saccades and fixations to generate patterns of movement.

Thesis Supervisor: Peter H. Schiller
Title: Dorothy W. Poitras Professor

ACKNOWLEDGEMENTS

Thanks to my family.

Thanks to Dr. Peter Schiller, besides for all the obvious reasons, for showing me every day how a neuroscientist should be: precise and tirelessly inquisitive.

Dr. Edward Tehovnik was my main collaborator on the work presented in this dissertation. I am very grateful for his patience as I learned all the techniques used in this study.

Thanks to Drs. Emilio Bizzi, Mriganka Sur, and James McIlwain who served on my thesis committee.

Thanks to Jan Ellertsen for trying to keep my life in order.

Thanks to the rest of the Schiller lab folks, especially to fellow graduate students Karl Zipser and I-han Chou. A million thanks to Warren Slocum for helping to keep my ancient PDPs working just long enough for me to finish this work. Thanks also to Wan-Ying Chee, Karen MacDonell, and Bobby Dolan.

Thanks to all my Boston-area non-MIT friends. You know who you are and I doubt any of you will read this anyway!

Thanks to my MIT friends, even though some tried their best to derail this dissertation with temptations of too much fun the last several months (names withheld to protect the guilty).

And thanks to Leslie Cameron, Ann Skoczenski, and Jay Edelman for being so cool that I decided to continue on in science.

CONTENTS

BIOGRAPHICAL NOTE

I was born in France, but no, I do not speak French. I grew up for 10 years in Roseville, Minnesota, then spent a couple years in Livermore, California, and then went to high school in Mobridge, South Dakota. I went to Texas A&M University for a year and then I transferred to Stanford University. At Stanford I received a Bachelor of Science degree in the double major of Biological Sciences (with Honors and with Distinction) and Electrical Engineering (with Distinction). I decided to better my understanding of information theory and signal processing, so I stayed another year at Stanford to get my Master of Science in Electrical Engineering. I have been at the Massachusetts Institute of Technology, in the laboratory of Dr. Peter Schiller, since September, 1990. Next year I will begin a post-doctoral position with Dr. Robert Wurtz at the National Institutes of Health in Bethesda, Maryland.

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Chapter 1

Introduction

A. Saccadic Eye Movements and Fixations

1. Saccades, fixations, and their graphic representations in this dissertation

Visual perception and eye movements are coupled in an exquisite manner. For example, as you read this page, your perception of the words flows in a smooth, nearly effortless manner. Usually, you are unaware of the many jerky eye movements you are making to accomplish this visual analysis. If you you notice a typographic error, however, the flow can become interrupted. Eye movements then become a conscious action as you try to locate the mistake (e.g. the multiple "you"s in the prior sentence) and start again. Why must eye movements be made in order for us to accurately perceive our visual surroundings? How does the brain accomplish the coordination between perception of visual input and the execution of eye movements?

Primates sense their visual surroundings with photoreceptors located in the retinas of their two eyes. Each retina has a small region, called the fovea, which contains receptors specialized for high acuity vision and color vision. To analyze a component of visual scene (e.g. a word on a page of text) in detail, the eyes must be moved in space so that the component's image is focused on the foveae. Since a natural visual scene can contain a rich variety of images that the primate might desire to

foveate, an alert primate's eyes are rarely still and normally move several times per second. The eye movements that are used to scan visual scenes are called "saccades" (from the French, meaning "to jerk").

Saccadic eye movements are very high velocity rotations of the eyes in the orbits. Both eyes are moved together in a yoked, or "conjugate", manner. By using saccades to move the eyes at hundreds of degrees of visual arc per second, the disadvantageous blurring of images on the retinas during the motion is brief. The ease at which primates move their eyes can lead one to presume that saccadic eye movements are a simple function. However, a surprisingly large amount of cerebral cortex, the cerebellum, and the brainstem are involved in the generation of saccades.

After a primate makes a saccade that causes foveation of a visual stimulus of interest, a period of "fixation" occurs. This means that the eye is held steadily at its position in the orbit. Fixation is an active process, requiring the six orbital muscles attached to each eyeball to remain taut at a constant length. Were the innervation of these muscles to disappear during fixation, the muscles would relax and the eyeball would resume its "primary position", with the pupil essentially centered in the orbit. Fixation of the eye does not imply a complete cessation of movement, it should be noted, but always involves a slight jittering of position and other "micromovements".

The research described in this dissertation investigates the

neural bases for the generation of saccadic eye movements and fixation in a laboratory animal, the rhesus monkey (Macaca mulatta). In particular, I and my colleagues examine the contribution of the frontal lobe of cerebral cortex to these oculomotor behaviors. The method we use is to temporarily inactivate areas of the frontal lobe using a local anesthetic and then observe how this affects the monkey.

A brief aside is warranted to describe how the movements of the eye in the orbit can be presented graphically. Although saccades and fixations are in fact rotational movements and positions, respectively, of two eyeballs, a convention in oculomotor research has been to transform these angular coordinates into a simpler two-dimensional (2-D) Cartesian coordinate system. The means by which I will display saccades and fixations is as follows. The position of one of the eyes is digitally sampled hundreds of times each second. I present these samples as dots in 2D Cartesian space (Figure 1). The horizontal location of the eye is plotted against the vertical location. A saccade therefore is shown as a dotted line illustrating the movement trajectory. One should think of this somewhat abstractly as describing the motion of the fovea across visual space. (Of course, this can be transformed back to the angular position of the eyeball in the orbit).

There are two fixation periods associated with every saccade: before and after the movement. Since very little movement occurs during a fixation, the many samples taken during

this period simply cluster at a nearly identical location in 2-D space. I replace these clusters with informative symbols. A cross marks the fixation that preceded a saccade, and a small square marks the endpoint fixation of a saccade (Figure 1).

2. Brainstem circuitry

Six muscles control each eyeball, the medial, lateral, superior, and inferior rectus muscles and the superior and inferior oblique muscles (Figure 2). The afferents of these muscles arise from three motor neuron nuclei in the brainstem. In order to control the length, and consequently the tension, in each muscle, the motor neurons employ a "pulse-step" method of discharge (Figure 3). The initiation of a saccade happens when the motor neurons relevant to the movement suddenly fire a pulse-like volley of spikes; this overcomes the inertia of the eyeball and causes it to begin rotation at high velocity. The pulse of a motor neuron's activity then slows to a tonic discharge, a certain step in frequency above or below what the cell's firing rate was prior to the saccade. This steady firing causes fixation to occur, as the muscles are held taut and the eye remains relatively still.

The motor neurons are controlled by regions in the pons. These areas contain cells which drive the motor neurons with pulse-like and tonic-like firing codes of different varieties (Figure 3).

A model of the brainstem saccadic generator circuitry was originally proposed by D. A. Robinson (1975), and it has been modified over the years by others (Figure 4). The role of the model is to show how the pulse-step temporal encoding might be generated. This is important, since the inputs to the brainstem are not coded in a pulse-step manner. Rather, they arise from the cerebral cortex and possibly the superior colliculus, neural structures that encode saccades in a spatial manner: the endpoint of the saccade is specified by a topographic locus of neural activity. Consequently, all models try to explain how the spatial information of the superior colliculus and cortex is transformed into the temporal code needed to drive the muscles appropriately. Modifications are often made to the models of the brainstem cirtuitry by authors in hopes of making sense of their nascent results; I will be a bit contrary to this tradition and not propose a new model. Rather, I will suggest that there is a common feature of all the models which can explain the significance of some of this dissertation's findings.

3. Eye fields of posterior cerebral cortex and the superior colliculus

The striate and extrastriate cortices are best known for their contribution to the analysis of vision, but given the level of coordination between vision and eye movements, it should not be surprising that they also have a direct oculomotor function.

Stimulation of the occipital cortex (which includes striate cortex, i.e. area V1) at high currents can elicit conjugate saccadic eye movements. This effect disappears if the superior colliculus is lesioned (Schiller, 1977), implying that the superior colliculus mediates the signals produced in V1 by electrical, and presumably sensory, stimulation.

Areas of parietal cortex are also involved in eye movements (for review see Andersen, 1989). Parietal cortex connects richly with areas of frontal cortex and with the superior colliculus, but its routes of oculomotor influence are still unclear. Keating, Gooley, Pratt, and Kelsey (1983) reported that stimulation-evoked saccades were abolished from parietal cortex following superior collicular ablations, a finding analogous to Schiller's (1977) result for occipital cortex. But later, this group modified their position (Keating & Gooley, 1988), claiming that the frontal lobe or its efferents also had to be lesioned to achieve the effect.

Among other functions, the superior colliculus and regions of posterior cerebral cortex are involved in the generation of "express" saccades (Fischer & Boch, 1983). Express saccades are seemingly reflexive saccades that are initiated after a reaction time of only 80 msec or so in the monkey (120 msec or so in humans). Following superior collicular lesions, express saccades are abolished (Schiller, Sandell, & Maunsell, 1987). The superior colliculus seems to receive its signal for the generation of express saccades from wide regions of the parietal

and occipital lobes and not from the frontal lobe (Schiller & Lee, 1992; Schiller et al., 1987).

Although lesions of the superior colliculus abolish express saccades, they do not seriously affect the production of longer-latency saccades. To cause devastation of saccade generation, collicular lesions must be combined with ablation of an area of the frontal cortex known as the frontal eye field (Schiller, True, & Conway, 1980) (Figure 5). Therefore, the frontal cortex contains at least one region vital for the production of eye movements. We now turn to a full consideration of the role of frontal cortex in oculomotor function.

B. Eye Fields of Anterior Cerebral Cortex

Ever since the electrical stimulation studies of Ferrier (1874), it has been recognized that the primate frontal cortex is involved in the generation of conjugate saccadic eye movements. Recently, it has been established that the frontal cortex is not homogenous in its contribution to saccadic behavior. There are at least two anatomically segregated regions involved with saccades. The first to be described was the frontal eye field (FEF), located in the anterior bank of the arcuate sulcus (Figure 6). A second region, the supplementary eye field (SEF), has been located medial to the FEF, near the hemispheric midline. Recent evidence suggests that the SEF is in the rostral portion of a larger eye and limb movement area, the dorsomedial frontal cortex

(DMFC) (Tehovnik, 1995) (Figure 6).

1. Electrical stimulation and single-unit recording studies

Frontal eye fields

Robinson and Fuchs (1969) were the first to examine the effects of electrical stimulation in the frontal cortex while the monkey's eye position was monitored precisely using the scleral search coil method. Their findings have been confirmed repeatedly since their initial report. Contraversive, conjugate saccades can be elicited with low (< 100 μA) currents from the FEF. They are evoked in a "vector" manner, in that they are of virtually identical amplitude and direction regardless of initial eye position. Prolonged stimulation of the FEF results in a sequence of nearly identical vectors, forming a "staircase" of saccades. The region that Robinson and Fuchs (1969) distinguished as the FEF ranged from the anterior bank of the superior and medio-inferior arcuate sulcus, up to the lip of the arcuate sulcus, and across the pre-arcuate sulcus region to the posterior half of the principal sulcus.

Bizzi (1968) and Bizzi and Schiller (1970) were the first to describe cells within this region that fire in association with saccades. These cells were 4 to 10% of the total sample, and they had post-saccadic responses. Later, Bruce and Goldberg (1985) re-examined the single unit responses of the FEF, using

different behavioral methods. Whereas the monkeys of the earlier Bizzi (1968) and Bizzi and Schiller (1970) studies were generating saccades at will, Bruce and Goldberg (1985) required their animals to execute saccades as a part of a rewarded task. With their techniques, Bruce and Goldberg (1985) found that over 50% of neurons in the FEF fire before visually guided saccades.

Other types of FEF cell responses were described besides those directly related to saccades. One important subset of the cell types is the class that responds during eye fixation. Bizzi (1968) found "Type II" cells, which responded during fixation, to be 5.7% of his sample. Bruce and Goldberg (1985) found that 7.0% of their cells responded to fixation or had foveal receptive fields. Although these fixation-related cells are a small part of the total FEF population, they contribute disproportionately to the subcortical efferents from the FEF. Using antidromic stimulation techniques, Segraves and Goldberg (1987) found that the majority of FEF cell types projecting to the superior colliculus were pre-saccadic movement cells and cells with foveal receptive fields or fixation-related activity. Other classes of cell type, such as those with peripheral visual receptive fields, were richly represented in the FEF but were found to have negligible contributions to its corticotectal projections. Similar findings were later reported for the FEF's projections to oculomotor regions of the pons (Segraves, 1992).

Bruce and colleagues (Bruce, Goldberg, Bushnell, & Stanton, 1985) attempted to define the anatomical extent of the FEF with

more precision than accomplished by Robinson and Fuchs (1969). By using a criterion that the core of the FEF was at sites that had a current threshold of < 50 μA, they confined the FEF to the anterior bank of the arcuate sulcus (Figure 6), at the conjunction of Walker's areas 8A and 45.

FEF cells that fire to head movements have also been described (Bizzi & Schiller, 1970). It is uncertain whether these cells would be included in the low threshold FEF of Bruce et al. (1985) or else lie rostral to it in the prearcuate gyrus. Modulation of FEF cell activity by other types of body movement has not been described, although it is unclear whether any group has looked for this specifically.

Evidence that the FEFs play a significant role in the generation of saccades therefore includes the ability to evoke vector saccades from the area at low currents, the presence of pre-saccadic activity in its units, and the projection of saccade- and fixation-related cells to the superior colliculus and pons. The qualitative nature of the FEF contribution to the signal that eventually reaches the brainstem saccadic generator is still unclear. Segraves and Park (1993) suggested that the instantaneous activity of FEF neurons is not related to the dynamic characteristics of saccades, in contrast to findings in the superior colliculus (Waitzman, Ma, Optican, & Wurtz, 1991). The timing of the onset of FEF firing is linked to the initiation of a saccade, and the FEF signal probably represents a vector for the saccade's trajectory. Goldberg and Bruce (1990) argued that

visual information is combined with eye position information in the FEF, so that the FEF signal represents a contraversive motor error vector, not the retinotopic position that needs to be foveated. In contrast, Dassonville, Schlag, and Schlag-Rey (1992) have used a "colliding saccade" method to illustrate that even ipsiversive saccades can be evoked from the FEF. They argue that a retinotopic goal signal, not a motor error signal, is sent downstream (Dassonville, et al. 1992). Regardless of the information content of the signal sent from the FEF to the tectum and pons, it is clear that it is sent in a vector fashion, either in motor or retinotopic coordinates. In other words, the FEF tells the oculomotor brainstem circuitry to increment the eye's position contraversively by a certain amount and angle; it does not tell the brainstem to move the eye to a specific, absolute position in the orbit.

Dorsomedial frontal cortex

Schlag and Schlag-Rey (1985, 1987) provided the first detailed demonstration that there is another eye movement field in the frontal cortex. They explored an area in the dorsomedial portion of frontal cortex, just lateral to the midline but significantly medial to the superior branch of the arcuate sulcus and thus removed from the FEF (Figure 6). Schlag and Schlag-Rey found that, as with the FEF, contraversive, conjugate saccades could be evoked from their "supplementary eye field" using low

currents and at short latencies. The electrically elicited saccades had an interesting distinguishing property relative to saccades evoked from the FEF: although stimulation of some SEF sites cause vector saccades, as found in the FEF, stimulation of many SEF sites caused saccades that converged toward a goal in visual space (i.e. caused the eye to rotate to a specific, absolute position in the orbit) (Schlag & Schlag-Rey, 1985, 1987). Another difference between the DMFC and FEF is that in the more caudal DMFC, forelimb reaching movements as well as eye movements can be evoked with electrical stimulation (Mann, Thau, & Schiller, 1988; Schall, 1991a); in contrast, FEF stimulation only causes eye movements.

Further study of the dorsomedial frontal cortex, which contains the Schlags' SEF in its rostral portion, has confirmed that saccades elicited from this region have more of a goal-directed nature than a vector nature (Mann et al., 1988; Mitz & Godschalk, 1989; Schall, 1991a; Bon & Lucchetti, 1992; Tehovnik & Lee, 1993). Only one study challenges this (Russo & Bruce, 1993), claiming that saccades elicited from both the FEF and SEF are intermediate between being vector and goal-directed. However, this is not conclusive for three reasons. First, Russo and Bruce (1993) used arbitrary stimulation parameters to elicit saccades from the SEF, instead of optimal ones (Tehovnik & Lee, 1993). Second, Russo and Bruce (1993) studied only the low current threshold SEF, which resides in the rostral DMFC. Eye movements can be evoked from a large region of DMFC caudal to the

classical SEF (Tehovnik & Lee, 1993), a region that Russo and Bruce (1993) ignored. Third, Russo and Bruce's explanation for their own findings cannot account for the fact that ipsiversive saccades can be elicited easily from the DMFC (Tehovnik & Lee, 1993; Tehovnik, Lee, & Schiller, 1994), but not from the FEF (excluding use of the colliding saccade paradigm of Dassonville et al., 1992).

Tehovnik and Lee (1993) found that there is a topography of head-centered goal sites located in the DMFC. Electrically stimulating the rostral DMFC causes saccades to converge to a far contralateral zone of termination. As the stimulating electrode is moved more posterior, the termination zone moves more ipsilateral. In the medio-lateral dimension, stimulating the lateral DMFC results in a saccade termination zone that is in upper visual space compared with stimulation of medial DMFC. Finally, both Schlag and Schlag-Rey (1987) and Tehovnik and Lee (1993) found another distinction between DMFC and FEF: staircase saccades are <u>not</u> elicited by prolonged train durations in the DMFC; rather, the eyes are driven into the goal area and do not move further even if stimulation is continued. Mann et al. (1988) discovered that DMFC stimulation can arrest eye movement, and Tehovnik and Lee (1993) later described this effect in more detail: stimulation while the eye is in the termination zone causes active fixation, since the ability to make saccades to visual targets is inhibited during such stimulation.

Single-unit recording studies have found that many DMFC

cells have a pre-saccadic response that can precede the saccade by > 300 ms (Schlag & Schlag-Rey, 1985, 1987; Mann et al., 1988; Schall, 1991a; Bon & Lucchetti, 1992). Another prominent class of cells responds during fixation, with or without a foveal receptive field (Schlag & Schlag-Rey, 1987; Schall, 1991a; Schlag, Schlag-Rey, & Pigarev, 1992; Bon & Lucchetti, 1992; Lee & Tehovnik, 1995). There are many other types of DMFC response, including those related to the preparation for movement (Schall, 1991a) and those related to limb movement and reception of juice reward (Mann et al., 1988).

A recent pair of papers (Chen & Wise, 1995a,b) used single-unit recording to demonstrate that the DMFC is a substrate for changes that occur during learning. These authors trained monkeys to make a certain direction of saccade when a visual stimulus was presented. During the training of this visuomotor association task, the firing of many cells in the DMFC was modulated in frequency (Chen & Wise, 1995a). In contrast, fewer FEF cells change their firing rate during the same learning task (Chen & Wise, 1995b). These findings support an earlier paper (Mann et al., 1988) that described plasticity of the DMFC using a combination of psychophysical training and electrical stimulation. Current work in the Schiller laboratory is examining the role of DMFC in learning in more detail, using arrays of chronically implanted microelectrodes to follow changes in neurons over periods of days.

The route by which the DMFC exerts its influence in the

generation of saccades is still unknown. The DMFC projects to many cortical and subcortical oculomotor structures, including the FEFs, the superior colliculi, the caudate nucleus, the central mesencephalic reticular formation, and the brainstem (Huerta & Kaas, 1990; Parthasarathy, Schall, & Graybiel, 1992; Schall, Morel, & Kaas, 1993). No investigators have yet reported studies using antidromic stimulation to determine the DMFC cell types that project to these areas.

Therefore, electrical stimulation and single-unit recording studies demonstrate that the DMFC is involved in the generation of saccades. One important difference between the DMFC and the FEF is that the DMFC seems to encode saccadic endpoints in a head-centered spatial map. In contrast, the FEF appears to relay a simple vector signal to its targets. Another difference is that the DMFC contains cells that fire in association with somatoskeletal movements, but the FEF does not. Electrical stimulation of DMFC can evoke either eye or body movements, but FEF stimulation evokes only eye movements. Finally, the DMFC appears to be more plastic in its ability to be modified through learning than the FEF. Despite this body of evidence, some investigators argue that there is not any significant anatomical (Mitz & Godschalk, 1989) or functional (Russo & Bruce, 1993) distinction between the two areas at all. Even among camps that acknowledge the differences between the two regions, there has been no clear consensus as to the distinctive roles of the DMFC and FEF. Since electrical stimulation and single-unit

recordings have failed to resolve the controversies surrounding the DMFC and the FEF, we must turn to the third major tool of systems neuroscientists: lesions.

2. Permanent lesion studies

Studies of the effects of permanent damage to the FEF have been done both in humans, subsequent to cerebral infarcts or after surgery for epilepsy, and in monkeys, following relatively precise aspiration lesions. Latto and Cowey (1971a,b) found a contralateral neglect and ipsilateral eye deviation after unilateral removal of monkey FEF. These were short-term effects, lasting only a few weeks. FEF lesions in the head-free monkey (van der Steen, Russell, & James, 1986) found that the head also suffered from ipsilateral deviation and contralateral neglect during this period. Schiller et al. (1980) quantitatively studied the oculomotor deficits following FEF and/or superior colliculus lesions in monkeys trained to scan an unchanging visual field. Surprisingly, they found that unilateral or bilateral FEF ablation caused only minor long-term deficits in their animals. Later studies (Schiller et al., 1987; Lynch, 1992) found that there was little effect of FEF ablation on saccades made to suddenly appearing targets, too. Deng and colleagues (1986) found the only known significant long-term deficit of unilateral FEF ablation: lesioned monkeys could not learn to make accurate saccades to remembered visual

positions in contralateral space.

Studies of human FEF lesions have found that the capacities for generating saccades toward remembered locations (Pierrot-Deseilligny, Rivaud, Gaymard, & Agid, 1991a; Pierrot-Deseilligny, Israel, Berthoz, Rivaud, & Gaymard, 1993; Rivaud, Muri, Gaymard, Vermersch, & Pierrot-Deseilligny, 1994) and for making "anti-saccades" into blank space, away from a visual stimulus (Guitton, Buchtel, & Douglas, 1985; Rivaud et al., 1994), are both impaired. Humans with FEF lesions make normal saccades to a flashed visual stimulus, however (Pierrot-Deseilligny et al., 1991b). Some frontal patients, in fact, cannot willfully suppress their reflexive eye movements to flashed visual stimuli (Guitton et al., 1985). These results suggest that, in the intact human, the FEF helps to generate voluntary saccades (those saccades that occur at will, not made as a simple reaction to visual input) and to inhibit saccades made reactively to a visual stimulus when such saccades are unwanted.

No studies in the monkey have quantitatively studied the oculomotor deficits incurred by DMFC ablations. But because bilateral lesions of the FEFs and superior colliculi render a monkey nearly completely unable to make any eye movements at all (Schiller et al., 1980), the DMFC cannot have an entirely independent, parallel influence in saccade generation. However, DMFC does not just project to the FEF alone or to the superior colliculus alone, since saccades can still be elicited from the DMFC after lesions of either the FEF or superior colliculus

(Tehovnik et al., 1994).

A pair of recent studies (Thaler, Chen, Nixon, Stern, & Passingham, 1995; Chen, Thaler, Nixon, Stern, & Passingham, 1995) showed that forelimb reaching movements were affected by DMFC ablation, but in a subtle manner: monkeys could still make correct reaching movements in response to visual stimuli, but they could not perform reaching tasks that required initiation and execution of movements in the total absence of cues.

Human lesion studies have found that DMFC damage does not impair saccades made during simple visually-guided or memory-guided saccade tasks (Pierrot-Deseilligny et al., 1991a,b). However, if the humans are required to make sequences of saccades, to a series of visual stimuli that appear, then a pronounced deficit is observed (Gaymard, Pierrot-Deseilligny, & Rivaud, 1990). Another study from the same group (Pierrot-Deseilligny et al., 1993) found that human DMFC lesions impaired the ability to make saccades in a craniocentric reference space: a target was flashed, the head was then rotated slightly, and the lesioned patients could not make saccades that compensated for the cranial displacement. FEF-lesioned and non-lesioned subjects could perform this task, however (Pierrot-Deseilligny et al., 1993).

Therefore, lesion studies complement the single-unit and electrical stimulation data in suggesting that the DMFC is involved in higher-level functions, such as the generation of sequences of eye or arm movements and the coding of saccades in

craniotopic coordinates. The FEF, in contrast, seems to be primarily involved in oculomotor functions, and in particular, those functions that require voluntary generation of single saccades in a vector manner. Because much of the DMFC and FEF lesion data are from human stroke victims, not precise surgical ablations, they suffer from imprecision in tissue localization and an absence of pre-lesion testing data. On the other hand, most surgical lesion studies in monkey find only temporary deficits in saccadic generation. Therefore, the specific roles of the DMFC and FEF remain unclear even after consideration of the permanent lesion literature. A new method, reversible inactivation of cerebral cortex, may provide the data needed to help resolve these issues.

C. Why Reversible Inactivation of the FEF and DMFC is Timely and Important

Studying the history of superior colliculus research provides insight into how we might make a significant advance in understanding the role of frontal cortex in saccade generation. It has long been known that either chemical or electrical stimulation of the superior colliculus produces conjugate eye movements (see Schiller, 1984, for a review). For over two decades it has also been known that neurons in the intermediate and deeper layers of this structure fire robustly in advance of saccades. Therefore, it was a mystery for years why lesions of

this area caused only minor effects. Schiller et al. (1980) suggested that the reason for this is that the FEFs and the superior colliculi form parallel pathways that converge at the pons. Therefore, following permanent surgical ablation of a single structure, other pathways and neural structures can take over the damaged structure's function to some extent. Bilateral ablation of the FEFs and superior colliculi is thereby required in order to achieve serious long-term oculomotor deficits (Schiller et al., 1980).

However, Hikosaka and Wurtz (1985, 1986) showed that inactivation of the superior colliculus alone was sufficient to cause a severe deficit in the ability to generate saccades to visual targets. This inactivation was done by injecting muscimol (Hikosaka & Wurtz, 1985) or lidocaine (Hikosaka & Wurtz, 1986) into the superior colliculus while the monkey was performing oculomotor tasks. This was not a permanent lesion, but was temporary, recovering within a few hours. This technique confirmed that the superior colliculus is indeed necessary for normal generation of visually-evoked saccades. Additionally it provided confirmation that the superior colliculus codes saccades in a topographic manner, since the loss of saccadic behavior was confined to a region of visual space predicted by the stimulation-evoked saccades evoked just prior to the injection. No one has yet reported any reversible inactivations of frontal cortical oculomotor regions.

In conclusion, reversible inactivation is timely because the

more traditional methods of single-unit recording, electrical stimulation, and lesion studies have failed to reveal the specific roles of FEF and DMFC in the generation of saccades and fixations. Currently there is a rather heated controversy over the respective roles of these structures. Reversible inactivation is important because it provides an elegant way to temporarily remove a component from the oculomotor system and study the result. I believe this method will become even more important to neurophysiologists and more commonly used once the physics of pharmacological inactivation become better understood. To this end we undertook experiments to determine the spread and time-course of inactivation following lidocaine before we even began the formal work of this thesis.

This dissertation reports on experiments in which I reversibly inactivated either the FEF or DMFC of awake, behaving rhesus monkeys. Chapters 2 and 3 relate the deficits incurred by reversible inactivation of the FEF and DMFC, respectively. A brief summary and discussion of the significance of these findings is presented in Chapter 4.

Legends for Chapter 1

FIGURE 1: Rotations of the eye in its orbit (left) can be represented as a 2-D trajectory in space (right). In this example, a rotation up and leftward by an angle of about 30 degrees (left) is transformed into an up and leftward trajectory (right). The trajectory on the right uses the following symbols: dots show the eye position at each sample time, a small cross shows the initial fixation position, and a small square shows the final fixation position (also called the saccadic endpoint).

FIGURE 2: Muscles of the eye and adjacent structures. (A) Lateral view. (B) Dorsal view. From Kandel, Schwartz, & Jessell (3rd Edition, 1991).

FIGURE 3: The pulse-step pattern of motor neuron firing (lower trace, Motor neuron) is formed from its phasic and tonic inputs from the pons (upper traces). The pulse causes the initial acceleration of the eye (bottom, Eye Movement position trace) and then the tonic firing maintains the eye at its new position. From Kandel, Schwartz, & Jessell (3rd Edition, 1991).

FIGURE 4: Basic brainstem saccadic generator circuitry model, adapted from Robinson (1981). Cerebral cortex, and possibly the superior colliculus (SC, Waitzman et al. 1991), send a desired

eye displacement signal (E_d) and a trigger signal to the pons. The trigger signal inhibits the omnipause neurons (OPN, see "Pause" trace of Figure 3) and allows the E_d signal to drive a saccade. The E_d signal is transformed into appropriate burst firings in the pons; this signal is integrated by the neural integrator (N.I.) and the burst plus integrated signal becomes the pulse-step input to the motor neurons. The integrated signal is fed back to cut down on the E_d signal and eventually end saccade generation.

FIGURE 5: Diagram of some of the brain's eye fields. Dashed line is cartoon outline of brain. The anterior cerebral cortex contains the FEF and DMFC, the structures examined in this dissertation. The posterior cerebral cortex contains the posterior parietal cortex (PPC) and occipital cortex (OccC), other areas from which saccades can be evoked. Subcortically, the superior colliculus (SC) is another eye field. Arrows show known connections between the areas. FEF and SC are emphasized in this figure, as they are vital nodes in the pathways from cortex to brainstem: bilateral ablation of FEF along with SC devastates saccade generation (Schiller et al., 1980; see text).

FIGURE 6: Photograph of a monkey brain, lateral oblique view. The DMFC regions are on either side of the hemispheric midline and the FEF is in the anterior part of the arcuate sulcus region. Scale in mm. Photo by Karl Zipser and Peter Schiller.

Figure 1

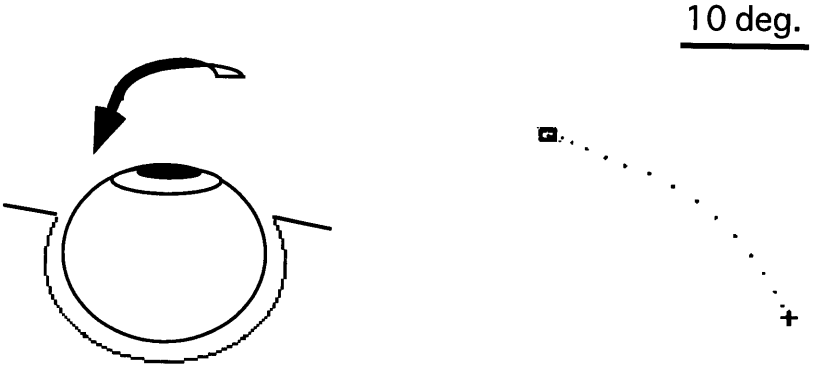


Figure 2

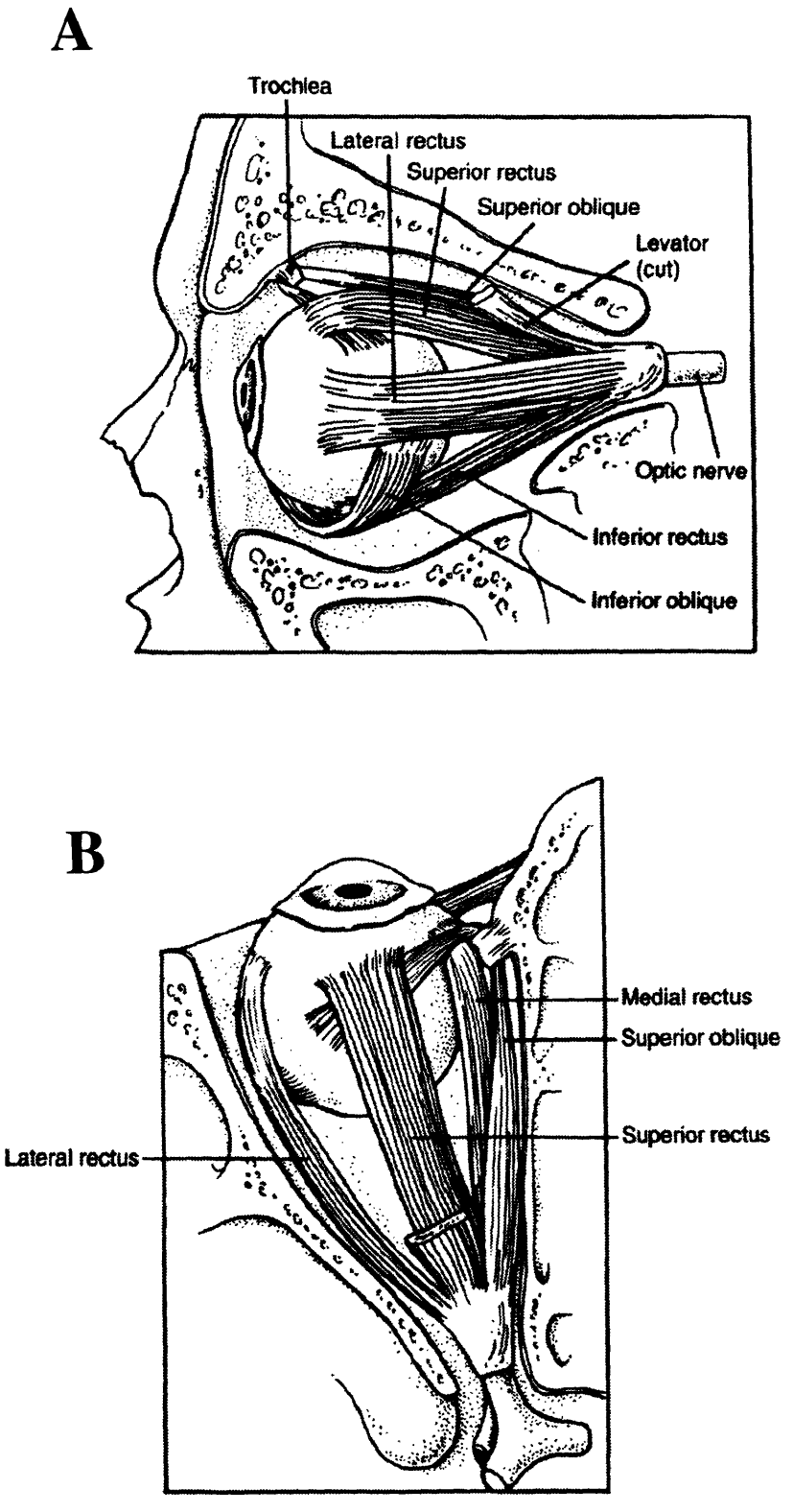

A
Trochlea
Lateral rectus
Superior rectus
Superior oblique
Levator
(cut)
Optic nerve
Inferior rectus
Inferior oblique
B
Medial rectus
Superior oblique
Superior rectus
Lateral rectus

Figure 3

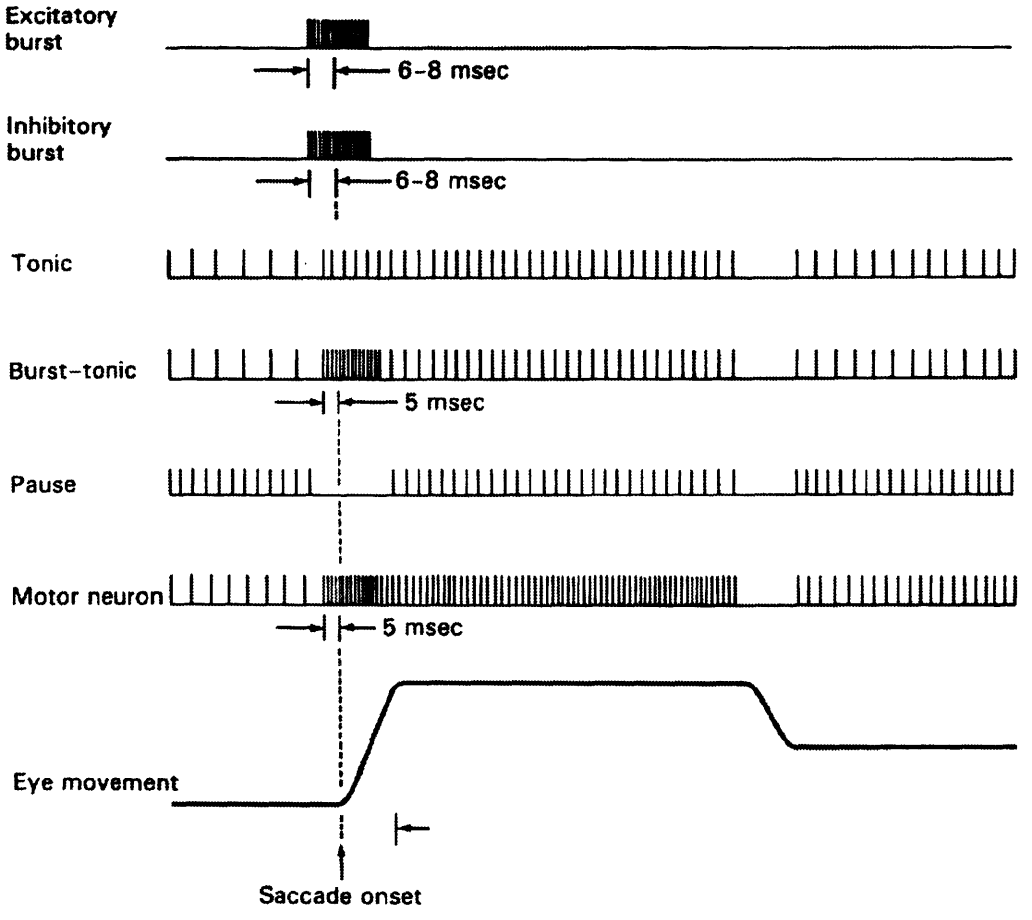

Excitatory burst
6-8 msec
Inhibitory burst
6-8 msec
Tonic
Burst-tonic
5 msec
Pause
Motor neuron
5 msec
Eye movement
Saccade onset

Figure 4

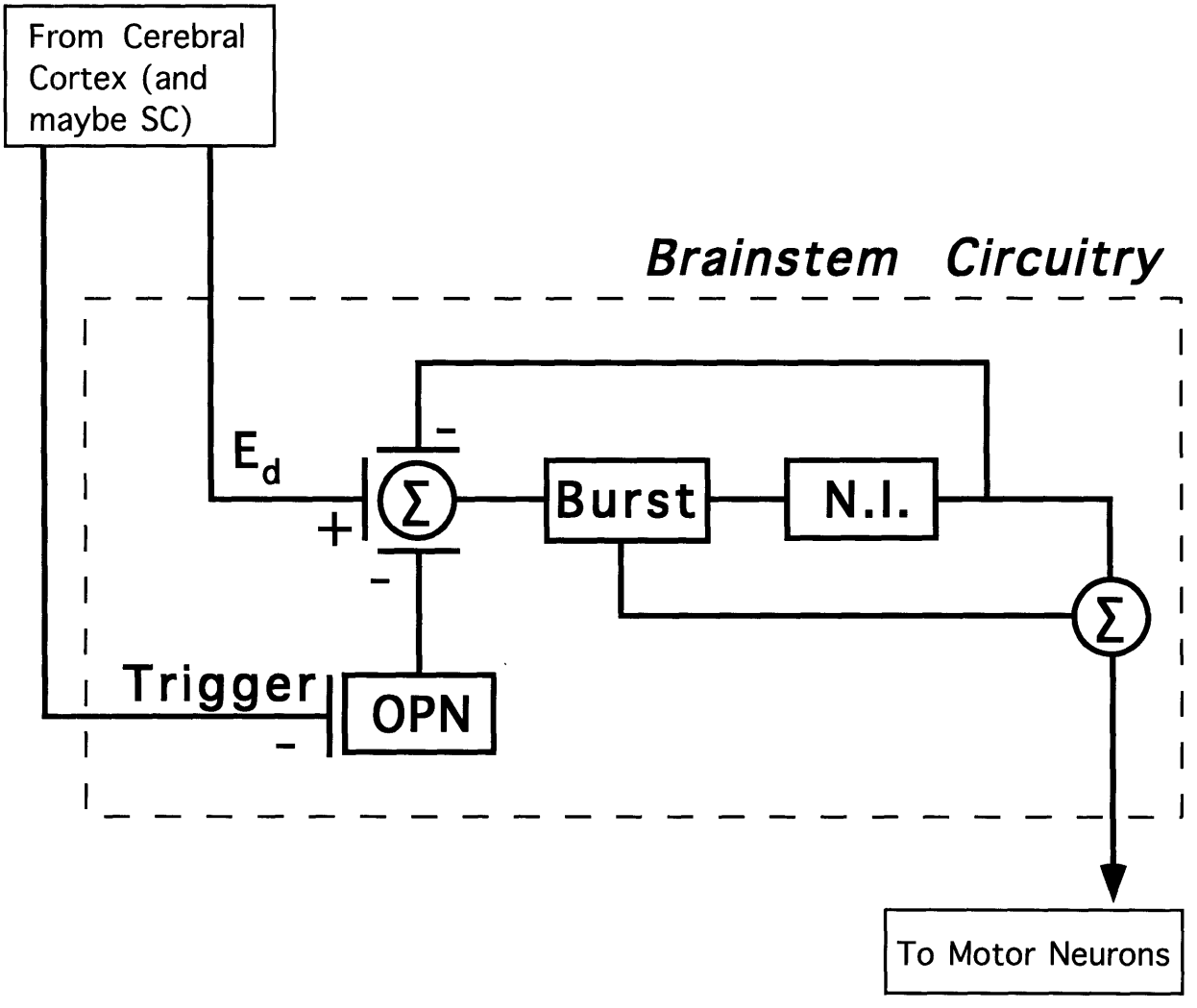

From Cerebral Cortex (and maybe SC)
Brainstem Circuitry
E_d
+
-
Σ
Burst
N.I.
-
Σ
Trigger
-
OPN
To Motor Neurons

Figure 5

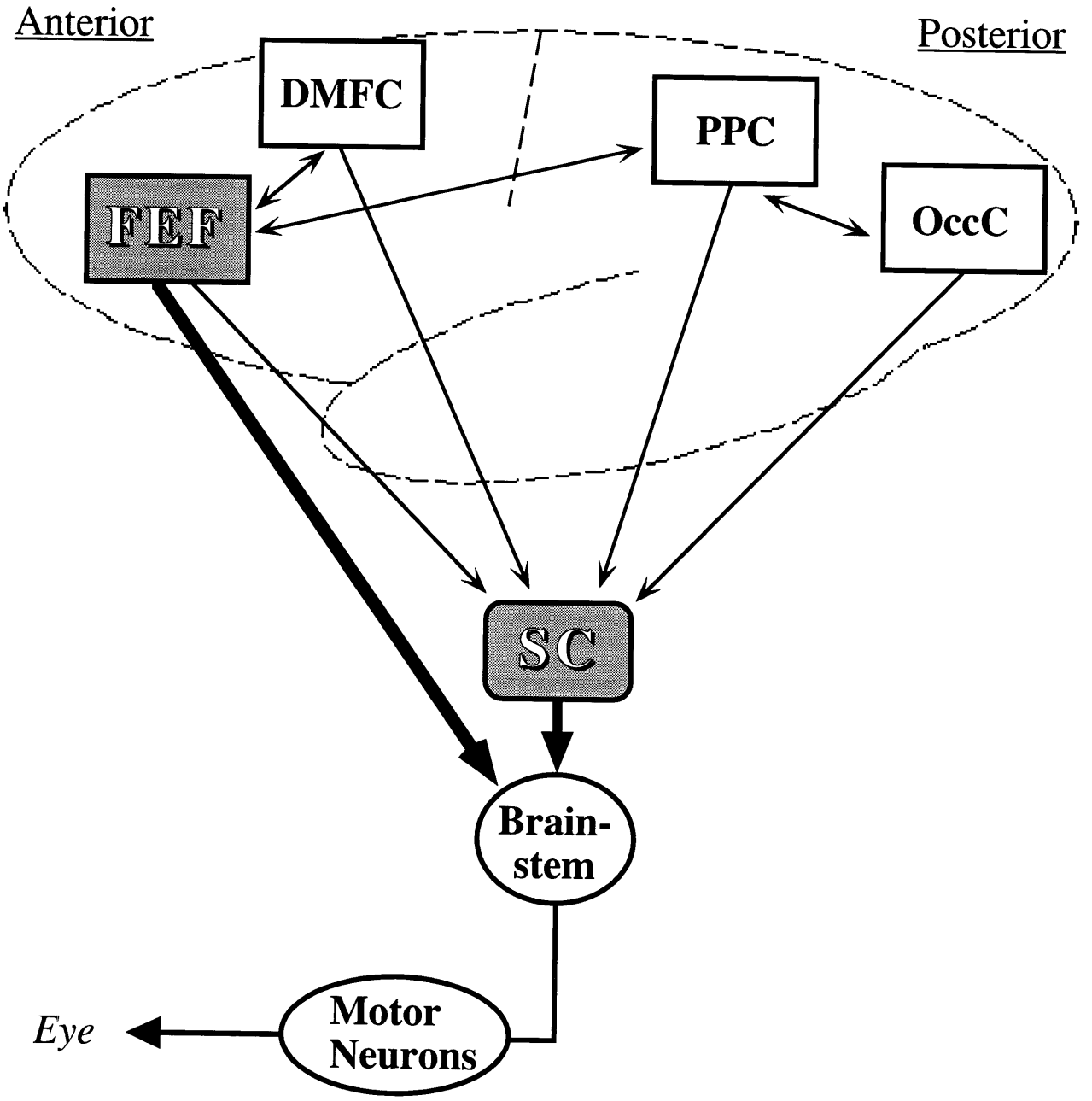
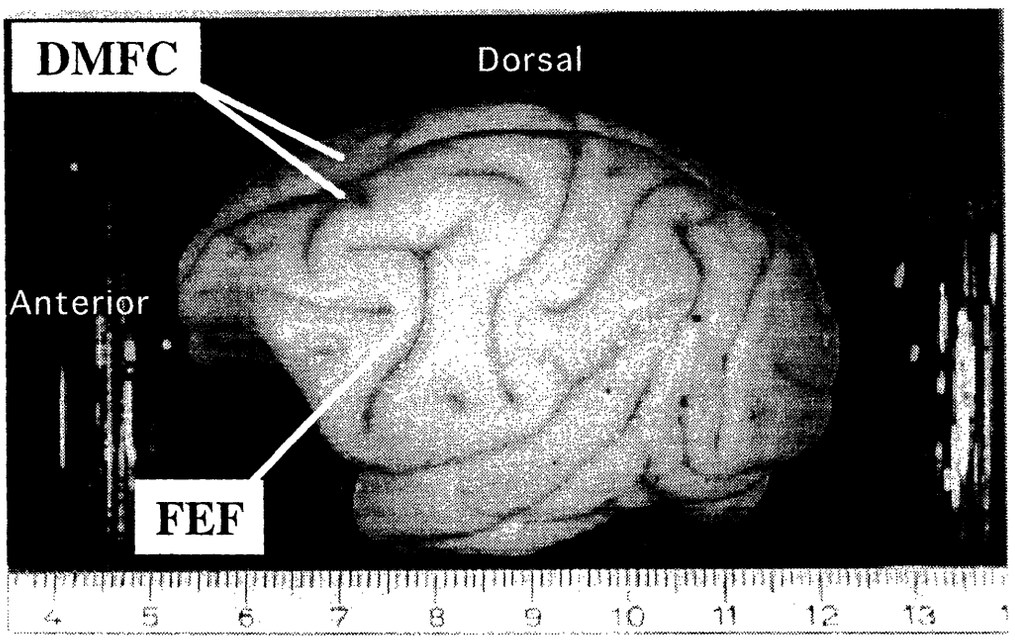

Anterior
Posterior
DMFC
PPC
FEF
OccC
SC
Brain-
stem
Motor
Neurons
Eye

Figure 6



Chapter 2

Reversible Inactivation of the Frontal Eye Fields

Introduction

The frontal eye fields (FEFs) might be involved in the generation of both saccadic eye movements and fixations. Strong evidence for this comes from single unit work (Bizzi, 1968; Bizzi & Schiller, 1970; Bruce & Goldberg, 1985), studies that used electrical stimulation to evoke behavior (Robinson & Fuchs, 1969; Bruce, Goldberg, Bushnell, & Stanton, 1985), and antidromic stimulation experiments (Segraves & Goldberg, 1987; Segraves, 1992). Permanent lesion studies, in surprising contrast, have provided less compelling evidence. Studies employing surgical lesions of the FEF in monkeys have revealed only mild long-term deficits in oculomotor behavior (Latto & Cowey, 1971a,b; Schiller et al., 1980; Schiller et al., 1987; Lynch, 1992), except for one report of an impairment in the accuracy of contraversive saccades to remembered target locations (Deng et al. 1986). Short-term deficits following FEF ablation include contralateral visual neglect, ipsilateral biases in fixation, and a decreased frequency of contraversive saccades (Latto & Cowey, 1971a,b; Schiller et al., 1980). These effects recover in a matter of weeks. Apparently, if the FEF performs a necessary oculomotor role in the intact monkey, its functions can be taken over by other brain areas after its removal. Human lesion studies have demonstrated moderate to severe long-term effects following surgical removal of, or cerebral infarcts within, the supposed

human FEF (Pierrot-Deseilligny, Rivaud, Gaymard, & Agid, 1991a; Pierrot-Deseilligny, Israel, Berthoz, Rivaud, & Gaymard, 1993; Rivaud, Muri, Gaymard, Vermersch, & Pierrot-Deseilligny, 1994; Guitton, Buchtel, & Douglas, 1985). These human findings are complicated by the encroachment of the damage into surrounding tissues and by the inability to obtain pre-lesion data on the subjects.

The purpose of this study was to see if there are any oculomotor deficits while a monkey's FEF is temporarily inactivated with lidocaine, an agent that binds to Na^+ channels (Ritchie, 1979; Ragsdale, McPhee, Scheuer, & Catterall, 1994). Temporary inactivation of FEF might reveal deficits that suggest the role of this structure in the intact oculomotor system. Advantages of this technique, compared with the technique of surgical ablation, are that 1) the inactivation of neural tissue lasts on the order of minutes, so there is little chance that other neural structures will compensate for the silencing of the FEF, 2) the FEF cells' activity can be monitored, so that one can correlate the shutdown and recovery of the FEF neurons with changes in the monkey's behavior, and 3) pre-inactivation, inactivation, and recovery data for an injection are all collected when the monkey is at the same, stable level of training. Disadvantages of reversible lidocaine inactivation are that 1) the exact amount of grey matter inactivated is hard to determine, and 2) the inactivation might involve adjacent white matter or non-FEF grey matter.

We addressed the disadvantages of reversible lidocaine inactivation as follows. First we undertook a pilot study to see how far inactivation caused by lidocaine spreads in the cortex. Therefore, by the time we began the formal behavioral experiments, we had a good understanding of the amount of grey matter affected by a lidocaine injection and we knew what time-course of inactivation to expect. Second, to address the possible complications of lidocaine inadvertently shutting down fibers of passage (because axons also have Na^+ channels), we checked our results by doing muscimol injections. Muscimol, a GABA agonist, only affects GABA receptors, which reside on cell bodies, not axons. Also, we carefully mapped out the FEF in our monkeys with electrical stimulation before we started the injections, to make sure we injected near the "core" of the FEF (Bruce et al., 1985).

We found that reversible inactivation of the FEF causes severe deficits in the generation of saccadic eye movements to remembered or briefly flashed contralateral visual targets. Also, FEF inactivation often causes impairment in the initiation and maintenance of contralateral fixation.

Methods

Monkeys

Two rhesus monkeys (Macaca mulatta) were used (designated as monkeys L and I). For surgery, a monkey was initially

anesthetized with ketamine (10 mg/kg) and then heavily sedated with pentobarbital (30 mg/kg). A scleral search coil was implanted subconjunctivally (Robinson, 1963; Judge, Richmond, & Chu, 1980) in the right eye. The skull was exposed and holes drilled and tapped, then screws were put in normal to the skull. Acrylic cement was applied, and a stainless-steel head post was set in the acrylic for use in restraining the head during experimental testing. In a subsequent surgery, a chamber was implanted to access the left FEF. It was centered approximately 20 mm lateral to the midline, at approximately +27 mm AP (the particular locations were chosen during surgery to allow a best fit of the chamber with respect to a pre-existing, adjacent chamber over the dorsomedial frontal cortex (DMFC)). The correct placement of the chamber could be verified visually, since the arcuate and principalis sulci could be identified through the dura. Monkeys were allowed several days to recover from surgeries before experimental testing, and they were placed on a regimen of antibiotics post-operatively to prevent infection.

The monkeys were deprived of water overnight before testing and they worked for apple juice reward during the experiments. They were allowed to drink to satiation following a day's testing. Throughout the testing food was freely available. The monkeys were provided for in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the guidelines of the MIT Committee on Animal Care.

Identification of FEF locations with stimulation mapping

The exact location and extent of an FEF within a chamber was assessed with electrical stimulation techniques. We had to balance two considerations when undertaking this stimulation mapping. First, the stimulation mapping was necessary because we wanted to ensure that we were aiming our injections near the "core" of the FEF, which has been defined as the region near the arcuate sulcus with current thresholds for electrical stimulation < 50 μA (Bruce et al., 1985). But second, like any invasive technique, the electrical stimulation mapping probably damaged the cortex to an extent, thus leaving us with slightly injured tissue into which to inject. Therefore, we kept our stimulation penetrations to a minimum. We determined some current thresholds in the region, we found the range of depths into cortex at which saccades could be evoked, and we noted the amplitudes and directions of saccades elicited.

This study was done in conjunction with a study of reversible inactivation of the DMFC. As assurance that both the DMFC and FEF regions were at a comparable level of integrity prior to beginning the injections, we used the same stimulation parameters in both eye fields. Since the DMFC requires specific ranges of train durations (generally >200 msec and < 800 msec) and pulse frequencies (100 to 200 Hz) (Tehovnik & Lee, 1993) for the optimal evocation of saccades, but the FEF does not require such constrained parameter ranges (Tehovnik & Sommer, submitted),

we used the DMFC-derived parameter set for stimulation mapping of both the FEF and the DMFC. These parameters were: cathodal followed by anodal biphasic pulse sequences, each pulse 0.1 ms in duration, pulse pairs occurring at 150 Hz, for a total train duration of 400 ms.

In both the FEF and DMFC we also tested many sites with parameters similar to those used by Bruce et al. (1985) and Russo and Bruce (1993), to see whether we were in the FEF or supplementary eye field (SEF), as defined by those studies, respectively. These alternative parameters were: cathodal followed by anodal biphasic pulse sequence, each pulse 0.2 ms in duration, 350 Hz pulse frequency, and 70 ms train duration.

In a FEF penetration, electrical stimulation was performed at the level of the first recorded unit, and then every 0.5 mm, usually until saccades could not be evoked any more. The "core" of the FEF was, as expected, in sites that provided access to the anterior bank of the arcuate sulcus, as surveyed by prior visual inspection during surgery. The < 50 μA FEF using Bruce et al.'s (1985) parameters corresponded to < 400 μA sites using our parameters. In monkey I we used seven adjacent sites, all of which had < 50 μA current thresholds using Bruce et al.'s (1985) parameters (Figure 1). In the other monkey (L), we used two sites with < 50 μA current threshold and six sites within 2 mm of these; all of these sites had < 400 μA current thresholds using our parameters. Sites adjacent to this cluster had > 400 μA current thresholds or were inaccessible due to dural tissue

growth and were not used.

Saccades were only evoked from the FEF once the electrode tip was 1.5 mm or more below the first unit. Saccades could be evoked until approximately 8 mm below the first unit. These depth results were methodologically informative for the subsequent injections, since we wanted to place the needle tips in the same depth range from which saccades could be evoked. Also, the depth data provided confirmation that the electrodes were descending into the bank of the arcuate sulcus. The evoked saccades were of medium amplitude, from 5 to 20 degrees, nearly all possible contraversive directions could be elicited in a penetration, and "staircase" saccades were evoked by prolonged stimulation trains; these attributes are typical of FEF (Robinson & Fuchs, 1969; Bruce et al., 1985).

Apparatus for injections

Our apparatus allowed us to slowly inject lidocaine, muscimol, or saline at a site while monitoring the nearby neural activity (Figure 2). The same apparatus was used in the pilot study (see below) and the formal study. The cut end of a 30-gauge needle was epoxied into the end of a 28 gauge cannula, so that 16 mm of the needle, from its cannula insertion to its beveled tip, was exposed. PE 50 tubing was fit snugly over the other cannula end. About 3 feet of tubing was run to a 100 μl Hamilton syringe, which was fixed in a slow injector. In

parallel to the needle-tipped cannula was a microelectrode for recording multiunit activity. A hydraulic microdrive assembly held the microelectrode and needle so that both moved in concert. The needle-electrode tip separation was approximately 1.5 mm.

The tubing was filled with distilled water except for the pharmacological agent at the needle end; a small bubble separated the two liquids. Monitoring of the bubble's position was used to double-check that the correct amount of lidocaine, muscimol, or saline was entering the brain during the injection. The needle and electrode tips were driven through the dura into the brain with the hydraulic microdrive.

Pilot study

In a pilot study we determined the relationship between the volume of lidocaine injected and the resultant spread and time-course of neural inactivation. This was done in the DMFC of a monkey that was not used for the present study. Over 70 injections were made with various volumes, using 1 or 2 mm separations between the needle and electrode tips. Injection rate was constant at 4 μl/minute. If a sufficient volume of lidocaine was infused, multiunit activity dropped within minutes after the start of an injection, it remained low for approximately 20 minutes, and then it recovered in a generally linear manner (Figure 3 A). The probability of shutdown increased with the amount of lidocaine injected (Figure 3 B).

Saline injections had no effect (Figure 3 C).

To quantify the neural inactivation, we defined shutdown as occurring if the multiunit activity dropped to less than 20% of its baseline level after start of the injection. The probability of shutdown at 1 or 2 mm from the needle tip as a function of lidocaine volume was calculated (Figure 3 D). Interpolating between the 1 and 2 mm radius results, we found that 18 μl of lidocaine could be expected to shut off neurons 1.5 mm from the needle tip 100% of the time.

In the present study we used a 1.5 mm separation, and hence a 18 μl lidocaine infusion volume, for two reasons. A practical reason was that we wanted the needle and electrode tips to be near each other, but not so close as to depress the dura excessively on the way down to the brain. Separation of 1.5 mm was found to work well. A theoretical reason for the choice of 1.5 mm was that cortex is approximately 3 mm thick and we wanted to attempt inactivating the entire thickness of cortex if possible.

Lidocaine injection protocol

The average depth at which saccades had been evoked in the preliminary stimulation mapping was used to guide the depth of needle tip placement. For example, for the FEF of monkey L, saccades were usually evoked from 3 to 5 mm below the first recorded unit. Therefore, we lowered our needle tip to this

depth range for injecting lidocaine. For monkey I we aimed the needle tip at 4 to 7 mm below the first unit.

The average multiple-unit firing rate during a trial was always measured with a microelectrode (glass-coated, PtIr, impedance 0.2 to 1.0 MΩ), as in the pilot study, to ensure that lidocaine was indeed being successfully delivered to cortex. This was considered crucial so that we could interpret possible negative results, i.e. if no behavioral effect was observed we needed to know that the FEF was indeed inactivated. In practice in these formal FEF experiments, the multiunit firing nearly always plunged to 0 Hz within 2 or 3 minutes after the start of the injection.

Muscimol injection method

Muscimol injections were performed with the same apparatus and using the same techniques as for the lidocaine injections, except for the following differences. The 100 μl syringe was replaced with a 25 μl Hamilton syringe, for a volumetric resolution of 0.5 μl. Markings were made on the PE tubing every 1 μl (this corresponds to approximately 4 mm of tubing), so that we could monitor movement of the bubble between the loaded muscimol and the distilled water that filled the rest of the tubing. Muscimol (5-Aminomethyl-3-hydroxyisoxazole, Sigma) was used at a concentration of 2 μg/μl, dissolved in sterile saline. A total volume of 2 μl was injected over a period of 13.5

minutes. The monkey's oculomotor behavior was checked periodically (for at least 10 minutes every half hour) for about four hours after the start of the injection. Because of the length of muscimol's effects, recovery data was collected the following day.

Visual stimulation and data collection

Visual stimuli were produced by light-emitting diodes (LEDs) fixed in a large board. This board was curved quasi-parabolically to minimize differences in luminance caused by LED angle. Sixty-three LEDs were mounted in the board, in 7 rows of 9. The monkey sat 108 cm from the board, and the LEDs were spaced by 5 degrees of visual angle to cover a total area of 40 deg. horizontally and 30 deg. vertically. Typically, 20 of the LEDs in a 5 x 4 array were used as visual targets (each separated by 10 deg.), and 3 of the LEDs on the horizontal meridian (20 deg. ipsilateral, central, and 20 deg. contralateral) were used as fixation points. Fixation LEDs were red and target LEDs were yellow.

The experiments were controlled by a program on a PDP-11 computer. The microelectrode signal was amplified (BAK, A-1B) and run through a window discriminator (BAK, DIS-1), and Schmitt trigger signals corresponding to the multiunit action potentials were sent to the PDP-11. The microelectrode signal was also viewed on an oscilloscope and fed to an audio monitor (Grass,

AM8B). The window discriminator levels were set above the noise level to pick up spikes that were obvious by visual and auditory means. The computer also collected eye position data and the timing of task events, at 333 Hz. Saccades were detected on-line using a 50 deg./sec velocity criterion.

Oculomotor tasks

Three tasks were used to assess the monkey's oculomotor abilities before, during, and after the lidocaine injections (Figure 4). The monkey performed all three tasks in total darkness (aside from LED glow during the tasks). This was verified once by a human observer who remained in the monkey's room for over an hour while the monkey ran tasks. A set of trials lasted 8 to 30 minutes, depending on the task and the severity of a monkey's deficit. The room light and the entire array of LEDs were turned on between sets of trials to keep the monkey alert and not dark-adapted. Any time the monkey seemed to be drowsy we paused the trials and made loud noises or flashed some lights. The monkey was occasionally given breaks of 5 to 15 minutes in the light every hour or so to prevent such drowsiness.

Both the eye position and eye velocity were used to drive the state system and synchronize events. For example, the computer judged that the monkey was looking at an LED if two conditions were met: the eye position was within an electronic window around the LED position and also a fixation occurred, i.e.

eye velocity fell below 50 deg./sec.

Memory target task: This task was used to see if the monkey could make saccades to targets if a response delay was imposed, such that the saccade had to be made to a remembered location (Figure 4 A). Three possible initial fixation positions (ipsilateral, central, and contralateral) and 20 or more possible target locations were randomized by trial. After foveation of a fixation LED, a target LED was lit for 300 ms and then extinguished (Figure 4 A). After another 300 ms, the fixation LED disappeared, and the monkey had to make a saccade to the remembered target location. A trial was aborted, with no reward given, if the monkey made a saccade at any time before fixation LED's disappearance.

The timing of this task was selected as a balance of two considerations. The total delay from target onset to fixation LED offset was 600 msec, which was long enough to enable us to confidently differentiate, by saccadic latency, between true memory-guided saccades and saccades triggered inappropriately by the target. On the other hand, the memory period of 300 msec was short enough to keep the spatial errors of the memory-driven saccades reasonably small (White, Sparks, & Stanford, 1994).

A memory saccade had to land within a 10 by 20 deg. target window for reward to be guaranteed. In cases of severe deficits, incorrect responses were sometimes rewarded to keep the animal from quitting. The target's window was rather large due to the inevitable upwards drift seen when testing monkeys in complete

darkness.

Visual target task: This task was used to measure the monkey's ability to make saccades to visual stimuli with no required delay. As with the memory target task, usually 3 initial fixation positions and 20 target locations were used. A variant of this task used only the central fixation position and randomized the target's duration 5-fold from 10 to 1000 msec. A fixation LED was lit to start a trial (Figure 4 B) and 100 msec after the monkey fixated it, it disappeared. If a saccade was made after fixation LED offset but before target LED onset, the trial was aborted. Otherwise, a target LED was lit 100 msec later. The monkey had to make a saccade directly into the 10 x 20 deg. target window and then fixate there in order to be guaranteed a reward. Incorrect trials were sometimes rewarded, in the cases of severe deficits.

Fixation task: This task was used to test the monkey's ability to acquire and fixate a visual stimulus for a relatively long period of time. In this task, only one LED was illuminated per trial. This LED was chosen randomly from an array of either 9 or 20 possible LED locations, spanning the entire 40 deg. x 30 deg. space. The monkey had 5 seconds to acquire the LED (i.e. enter its window and fixate there) and then he had to keep his eye position within the window for an additional 5 seconds (Figure 4 C). The animal received twice as much juice for staying within the window for the full 5 seconds as it did for simply acquiring the LED and then leaving the window.

Analysis

We recorded eye position throughout the trials. Trial events, LED positions and window sizes, and the average multiunit firing rate during the trial were also recorded. Only the first saccade made after target onset was analyzed. Saccades of amplitude ≤ 2 deg. were indistinguishable from fixation-related "micro-movements" and were not analyzed, but their frequencies of occurrence were noted. For the first saccades made that were > 2 deg. in amplitude, the following analyses were performed. Saccadic error was defined as the distance from the saccadic endpoint to the actual target location. Saccadic latency, amplitude, and peak velocity were also calculated. 2-D plots of saccadic trajectory were made of all data sets for qualitative inspection. The following considerations were specific to each task:

Memory target task: Trials that were aborted before the target was presented were not analyzed. Trials that were incorrect due to the monkey looking prematurely at the target, before the fixation spot disappeared, were analyzed. Saccadic latency for these premature responses was with respect to target onset. Correct trials, in which the monkey waited until the fixation spot disappeared before making a saccade, were also analyzed. Saccadic latency for correct trials was relative to fixation spot offset.

Visual target task: Trials aborted before target onset were

not analyzed. All other trials were analyzed. Saccadic latency was with respect to target onset.

Fixation task: There were no aborted trial types in this task; every trial was analyzed. Three primary indices of fixation ability were quantified, and then a secondary, overall, fixation index was calculated. "Acquisition percentage" was calculated as the percent of times that the monkey was able to enter and fixate within an LED's window when that LED was lit, within the 5 sec. time limit. "Acquisition time" was the latency from LED appearance to fixation within that LED's window. "Fixation error" was the average error, in deg., of the eye position with respect to the target LED's location, during the required 5 sec. fixation time. This was calculated by finding the error every 3 msec after acquisition and then calculating the average over all 1665 samples (333 Hz x 5 sec.). The overall "Fixation Deficit Index" was meant to take all three of these primary measures into account at once, and was derived as (Mean Fixation Error) x (Mean Acquisition Time) / Acquisition Percentage. Hence, larger Fixation Deficit Indices signified more severe inabilities to fixate visual stimuli.

Inter-trial Period, in the Dark: Finally, in every injection we measured the eye's position between trials, when the monkey was resting in complete darkness. One fixation location during each inter-trial interval was stored, and in off-line analysis all these fixation locations were superimposed and the mean and standard deviation of the scatter plot were calculated.

This was to determine if the injections caused any deviation of the eye's resting position in the absence of visual input.

Results

Overall injection results

A total of 20 injections were made into the FEFs of the two monkeys. Thirteen of these were lidocaine injections (9 in monkey L, 4 in monkey I). Four saline injections, of equal volume as the lidocaine injections, were made in monkey I. Two muscimol injections were performed in monkey I, and one saline injection of equal volume as the muscimol injections was also done in this animal.

The locations of sites used during experiments with the three tasks are illustrated in Figure 1. Some of the sites were used for more than one injection.

Memory target task

Inactivation of the FEF caused severe deficits in the ability to generate contraversive saccades to remembered target locations. This was found in 3 out of 3 lidocaine injections and verified with a muscimol injection. A saline injection to match the lidocaine injection volume caused neither a neural shutdown nor a behavioral deficit.

Figure 5 demonstrates this effect. Before the injection began, the FEF multiunit firing was moderate and stable, and the monkey could make saccades in all directions (Figure 5 A). Just after the lidocaine was injected, the FEF multiunit firing dropped, and the monkey was severely impaired at making saccades into contralateral space (Figure 5 B). Responses to contralateral targets either were not present, were ipsilateral, or were nearly vertical. The multiunit firing began to recover after 30 minutes. Two hours after the injection both the neural firing and the saccadic behavior had nearly fully recovered (Figure 5 C).

Both the saccadic error and the saccadic latency were increased significantly for contralateral targets during the FEF neural inactivation (Figure 6). This was true for lidocaine as well as muscimol injections (Figure 6 A,B,C), but it was not true for saline injection (Figure 6 D). The effect from muscimol injection had a later onset (approximately 60 minutes after injection) and much longer duration (over 4 hours, at which time testing was discontinued). This time-course is not unusual for muscimol injection (Hikosaka & Wurtz, 1985; Schiller et al., 1987). Recovery data for muscimol injection were collected on the following day.

The frequency of premature responses, those made after target onset but before the fixation offset, increased dramatically during FEF neural inactivation, for ipsilateral target presentation only (Figure 7). This effect was sometimes

accentuated if the eye was initially fixating contralateral (thin solid line in Figure 7). The effect had a later onset for muscimol injection (Figure 7 C). A saline injection used to match the lidocaine injections showed no effect and it was discontinued after an hour (Figure 7 D). The premature responses had normal latencies with respect to target onset (from 150 to 350 msec).

Visual target task

During FEF inactivation, monkeys were severely impaired in their ability to make saccades to briefly flashed contralateral targets in the visual target task. In contrast, saccades made to long duration contralateral targets were only mildly affected. Five out of 5 lidocaine injections, and a muscimol injection, confirmed this. Two saline control injections that matched the lidocaine injections, and 1 saline injection that matched the muscimol injection, resulted in no behavioral effect.

An example of an injection experiment in which a 30 msec duration target was used is shown in Figure 8. Like in the memory target task (cf. Figure 5), contraversive saccades were nearly totally eliminated while the FEF neurons were shut down (Figure 8 B). Before and after the neural inactivation, contraversive saccades could be generated (Figure 8 A, C).

Figure 9 shows the continual time-course of the deficits during this experiment in relation to the time-course of neural

inactivation. Each datum is the error (Figure 9 A) or latency (Figure 9 B) of a single saccade. Saccades made in response to contralateral target presentations have elevated errors and latencies just after the lidocaine injection. The errors and latencies decrease steadily as the neural firing increases (Figure 9 C). The results shown in Figure 9 were typical of all the visual target task results (using brief target flashes) and the memory task results.

Two experiments in which long-duration targets (1000 msec) were used, rather than the brief 30 msec target flashes, revealed only mild deficits (not shown). Therefore, to examine this difference more rigorously, a series of experiments in which the target duration was varied from 10 to 1000 msec, randomly by trial during the same injection, was performed.

The first effect of target duration revealed by this series of experiments was that, while the FEF was inactivated, often the monkey's first saccadic response to brief contralateral targets was either excessively delayed, such that it was absent from that trial's data, or it was unusually small in amplitude (Figure 10). These failures to make a significant-amplitude saccade to the target were most common when the target was very brief: for 10 and 30 msec target durations during FEF inactivation, about 30% of trials were of this type.

For those trials in which a significant-amplitude saccade was made after the target was flashed, the saccadic errors and latencies of these responses were quantified. Saccades to brief

contralateral target flashes (10, 30, 100, and 315 msec) had significantly increased error (Figure 11) during the FEF inactivation. In contrast, saccades made to long duration (1000 msec) contralateral targets were not significantly dysmetric during FEF inactivation (Figure 11). This target duration effect was true for both lidocaine (Figure 11 A,B) and muscimol injections (Figure 11 C); saline injection had no effect (Figure 11 D). Saccadic latencies were also increased to contralateral flashed targets during FEF inactivation; this latency deficit affected all target durations (Figure 12).

Orbital effects

For many of the injections we examined the influence of the eye's initial orbital position on the deficits. Figure 13 illustrates this for the data previously shown in Figure 8. During this injection the initial eye position was actually randomized three-fold (only the central fixation cases were shown in Figure 8); the fixation spot either appeared 20 degrees ipsilaterally, centrally, or 20 degrees contralaterally. As was illustrated in Figure 8, FEF inactivation caused a severe deficit in the generation of contraversive saccades when the eye was initially fixating centrally (Figure 13, middle row). But if the eye were initially in ipsilateral space, some contraversive saccades could indeed be generated, although they were significantly foreshortened compared to before the inactivation

(Figure 13, top row). This was typical of all the experiments in which the orbital position of the eye was varied during FEF inactivation, including the memory target task experiments.

The orbital effect is quantified in Figure 14 for the same experiment as shown in Figure 13. The overall saccadic error and latency during FEF inactivation is nearly identical for contraversive saccadic attempts made from ipsilateral versus central fixation (Figure 14, column A versus column C). If the retinotopic location of targets from ipsilateral fixation is matched with those used in central fixation (column B), the deficit in error and latency from ipsilateral fixation is still significant, but with a higher p value (Column B: saccadic error, $t(13)$, $p = .0054$; saccadic latency, $t(13)$, $p = .0019$. Column C: saccadic error, $t(11)$, $p = .0007$; saccadic latency, $t(11)$, $p = .0007$).

Fixation task

In 2 out of 3 lidocaine injections, the monkey had a severe inability to acquire and fixate visual stimuli in peripheral contralateral space. A saline injection to match the lidocaine injection caused no deficit. This inability to acquire contralateral fixation lights was also commonly seen in those experiments that varied initial fixation position of the eye to test orbital dependence of effects. Often, testing was difficult because the monkey could not fixate the contralateral fixation

point reliably while the FEF was inactivated.

Acquisition percentage decreased, acquisition time increased, and fixation error increased for attempts to foveate targets in contralateral space for the two effective lidocaine injections (data from one is shown in Figure 15). The overall measure, the Fixation Deficit Index, took all these three factors into account and showed a large and highly significant increase for the two effective lidocaine injections (Figure 16 A,C), a mild increase for the other lidocaine injection (Figure 16 B), and no effect for the saline injection (Figure 16 D).

Although contralateral space was the most highly affected, in two cases lower visual space, even into the ipsilateral hemifield, was also slightly affected (Figure 16 B,C). Finally, the inability to maintain fixation, as measured by fixation error (Figure 15 D), was due to saccades and drifts made away from the fixation light once it was acquired; nystagmus was never observed.

Inter-trial intervals

During the inter-trial interval, the animal was in darkness. In every one of the 13 lidocaine injections there was a sharp ipsilateral shift in the eye's resting position during the inter-trial interval, just after the FEF was inactivated (Figure 17 A, thin lines). An ipsilateral shift was seen in only 1 of 4 saline injections (Figure 17 A, thick lines). An ipsilateral shift was

seen in both of the muscimol injections, but it developed over a longer time course (Figure 18 A, thin lines). There was no ipsilateral shift seen in the saline injection used to match the muscimol injections (Figure 18, thick line).

The average ipsilateral shift in the dark just after FEF inactivation with lidocaine was -5.91 degrees (SD 1.92 deg). This was a highly significant change in position (from -6.86 to -12.77 deg.; $t(12)$, $p < .0001$). For comparison, during the saline injections the average horizontal shift was only -0.093 degrees (SD 3.07 deg.), which was insignificant (from -6.02 to -6.11 deg.; $t(3)$, $p = .4778$). Before injection, the average horizontal eye position was indistinguishable for the saline and lidocaine experiments ($t(3)$, $p = .3405$), but just after injection the populations did not overlap at all and were significantly separated (Figure 17 A) ($t(3)$, $p = .009$).

There was also a trend for the vertical eye position in the dark to shift downward, but this was seen in lidocaine and saline injections alike (Figure 17 B) as well as for the muscimol injections (Figure 18 B). The average downward shift for lidocaine injections immediately following the injection was -1.64 deg. (SD 1.89 deg.) (from 6.66 to 5.02 degrees) and for saline injections it was -0.78 deg. (SD 1.37 deg.) (from 9.35 to 8.57 degrees). The downward shift was more significant for lidocaine injection ($t(12)$, $p = .0044$) than for saline injection ($t(3)$, $p = .0425$).

Saccadic dynamics

We examined the relationship between saccadic peak velocity and amplitude (the "main sequence") for contraversive saccades generated before and after vs. during the FEF inactivation. We examined saccades made to remembered or briefly flashed targets, since they were most affected by FEF inactivation. One complication was that a major deficit of shutting down the FEF was an inability to even make contraversive saccades to these targets, at least from initially central fixation (the "orbital effect"). Therefore, we examined saccades made to remembered or to briefly flashed targets from initially ipsilateral fixation. As the example of Figure 13 showed, most of these saccades had a small, but non-zero, contraversive component.

The main sequences derived by this method are shown in Figure 19. Saccades made to a briefly flashed target (30 msec duration) are used in Figure 19 A and B, and those made to remembered target locations are used in Figure 19 C and D. In general, the saccades made during FEF inactivation are of much shorter amplitude than those made before and after inactivation. There is a hint of a difference using the visual target task at these short amplitudes (Figure 19 A), and zooming in on the range from 2 to 12 degrees illustrates that the velocities in this range are significantly depressed by FEF inactivation (Figure 19 B) (slopes: 33.3 and 19.1 (deg./sec)/deg., $t(66)$, $p < .05$). Using the memory target task, we found that velocities are

generally lower than in the visual target task (note the difference in scale, Figure 19 A versus C), as has been demonstrated previously (White et al., 1994). A trend for depressed velocities at the lower amplitude range during the inactivation is present here, too (Figure 19 D), but the regressions are not significantly different (cf. Figure 19 B).

Discussion

Reversible inactivation of FEF caused saccadic deficits, fixation deficits, and other impairments. We will discuss the nature and possible reasons for each deficit.

Saccadic deficits

Saccadic tasks affected by FEF inactivation: While the FEF was shut down, there was severe impairment in saccadic accuracy to remembered target locations and to the briefer visual flashes. What is common to these two situations? For remembered target locations, the target is gone before the saccade is initiated. Likewise, for brief target flashes, the target typically disappears before saccade onset. In contrast, saccades can be made accurately to long-lasting visual stimuli during FEF inactivation. The common finding is that the FEF is necessary to generate visually-triggered contraversive saccades when the visual stimulus disappears before the saccade can be executed.

Is the deficit visual, motor, or memory? Previous authors have shown that FEF ablation causes visual deficits (Latto & Cowey, 1971a), saccadic deficits if the SC is also removed (Schiller et al., 1980), and memory deficits (Deng et al., 1986). Any lesion or temporary inactivation of FEF silences a variety of cell types: 40% of the FEF's presaccadic units have only visual activity, 20% have only movement activity, and 40% have both types of response (Bruce & Goldberg, 1985). The latter class of "visuomovement" cells often exhibit memory-like responses, continuing their firing long after a stimulus disappears (Bruce & Goldberg, 1985; Funahashi et al, 1989). We expect that our findings are due to a combination of visual, motor, and memory deficits. In the next section we propose a specific mechanism by which these three deficits might have caused our results.

FEF and the brainstem circuitry: E_d signal: Models of the brainstem saccadic generator have as inputs a desired eye position (or displacement) signal, E_d, and a trigger signal (Robinson, 1975; Van Gisbergen, Robinson, & Gielen, 1981; Jurgens, Becker, Kornhuber, 1981). E_d is presumed to be a long-lasting signal that requires cutoff by negative feedback from a neural integrator. It is not hard to imagine that the photic energy from a prolonged visual stimulus could drive such an E_d signal. But what if sensory input is transient?

For the remembered and brief target flash conditions, photic energy is not available to drive E_d throughout the saccade's generation. With such transient input, a neural representation

of the input must be maintained after the stimulus is gone. Such "memory activities" have been found in FEF (see above) and also in area LIP of the posterior parietal cortex (Gnadt & Andersen, 1988; Andersen Bracewell, Barash, Gnadt, & Fogassi, 1990) and in the dorsolateral frontal cortex (DLFC) (Funahashi, Bruce, & Goldman-Rakic, 1989). The FEF is heavily interconnected with both LIP (Huerta, Krubitzer, & Kaas, 1987; Andersen, Asanuma, Essick, & Siegel, 1990) and DLFC (Barbas & Mesulam, 1981; Huerta et al., 1987).

Ablation of DLFC impairs saccades made to remembered targets (Funahashi, Bruce, & Goldman-Rakic, 1993). Preliminary results suggest that reversible inactivation of LIP disrupts memory-guided saccades, too (Mazzoni, 1994). Saccadic signals from cortical structures such as DLFC and LIP require mediation through the FEF or SC (Schiller et al., 1980). Since an intact DLFC, LIP, and FEF are each necessary for generation of memory-guided saccades, these three structures might form a network for the translation of visual input into "memory activity", which the FEF can then send as a E_d motor command to the brainstem.

FEF and the brainstem circuitry: trigger signal: Besides the E_d signal, which determines the metrics of the next saccade, the cerebral cortex is presumed to supply a trigger signal to the brainstem. The trigger signal determines a saccade's timing by disinhibiting the omnipause neurons (see Robinson, 1981, for a review). The FEF seems to contribute to this trigger signal. During FEF inactivation, contraversive saccades made in all

saccade tasks had increased latency. For very brief target flashes (Figure 10), it was common for saccades to not be initiated at all.

This trigger function is unlikely to be unique to FEF. Increased saccadic latencies and decreased rates of saccade production have also been demonstrated following lesions of the posterior parietal cortex (Pierrot-Deseilligny et al. 1991b; Lynch & McLaren, 1989) and the superior colliculus (Schiller et al., 1980; Hikosaka & Wurtz, 1986).

Fixation deficits

We tested the monkey's ability to fixate because of Latto & Cowey's (1971b) finding of a bias for ipsilateral fixation in FEF-lesioned monkeys and Segraves' recent work showing that FEF cells with foveal visual fields project strongly to SC (Segraves & Goldberg, 1987) and to the pons (Segraves, 1992) despite their rarity in the FEF (Bizzi, 1968; Bruce & Goldberg, 1985). We found that FEF inactivation caused severe deficits in the ability to fixate targets contralaterally in most, but not all, of the experiments. All three measures of fixational ability, acquisition time, acquisition percentage, and fixation error, were affected.

At least two areas of cerebral cortex besides the FEF are involved with fixation. Cells in the posterior parietal cortex have long been known to carry fixation-related signals

(Mountcastle, Lynch, Georgopoulos, Sakata, & Acuna, 1975) and this region is heavily interconnected with FEF (Huerta et al., 1987; Stanton, Bruce, & Goldberg, 1995). The DMFC or supplementary eye field (SEF) also contains many cells with fixation-related activity (Schlag & Schlag-Rey, 1987; Schlag, Schlag-Rey, & Pigarev, 1992; Bon & Lucchetti, 1992; Lee & Tehovnik, 1995), and it too has reciprocal projections with FEF (Huerta et al., 1987; Huerta & Kaas, 1990; Schall, Morel, & Kaas, 1993). Therefore, the FEF may play a role in mediating the fixation-related signals of these two areas.

The effects of FEF inactivation on contraversive saccades, as described above, probably influenced the results from the fixation task. Contraversive saccades are required to get into contralateral space before fixation can even be attempted there. However, the LEDs in the fixation task were lit for at least 5000 msec. FEF inactivation only affects the latency, not the accuracy, of saccades to such long-duration targets. Therefore, saccadic deficits probably only contributed to the increase in acquisition time. Acquisition percentage and fixation error likely reflected true fixation deficits and not saccadic impairment.

Comparatively, fixation generation is probably a less important function of the FEF than saccade generation. Not all lidocaine injections caused a severe fixation deficit. Also, electrical stimulation of FEF never causes fixations, only saccades. Fixation can be evoked from other areas, such as the

DMFC (Tehovnik & Lee, 1993) and the rostral SC (Munoz & Wurtz, 1993b).

Other effects

Premature responses: The frequency of premature saccades in the memory task, inappropriately made to a flashed ipsilateral target when the monkey was supposed to continue fixating, was sharply increased during FEF inactivation. Similar inabilities to suppress inappropriate, "reflexive", saccades have been reported following permanent human FEF lesions (Guitton et al., 1985).

The premature responses are likely due to an interaction between 1) the fixation and saccadic deficits resulting from left FEF inactivation, and 2) the normal saccadic signals being generated by the right FEF. In the normal animal, the signals to maintain fixation probably inhibit the signals to make a saccade, e.g. through a feedback system in the SC (Munoz & Wurtz, 1993b), one of the structures that receives FEF input (Segraves & Goldberg, 1987). During FEF inactivation, fixation is impaired. Therefore, the saccade signals become disinhibited. Premature contraversive (rightward) saccades do not appear because the disruption of such saccades is an additional effect of (left) FEF inactivation. Ipsilateral targets recruit the other (right) FEF, however, which is providing its normal signal to the rest of the oculomotor system. Hence, premature responses occur and they are

ipsiversive (leftward). This interpretation is supported by the observation that the frequency of premature saccades was often worse for more contralateral fixation; this reflects the contralaterality of the fixation deficit.

Inter-trial interval eye shifts: The ipsiversive shift of eye position in the dark was probably another effect of the imbalance between the inactive left FEF and the normal right FEF. During FEF inactivation, the monkey cannot fixate well in contralateral space and is impaired at generating contraversive saccades. Therefore, in the dark the eyes are driven into ipsilateral space by the intact FEF (also suggested by Latto & Cowey, 1971b). Additionally we found a slight downward shift during FEF inactivation, but this also occurred with saline injections and might have been due to a factor common to all injections, such as fatigue.

Orbital effects: A monkey with an inactivated FEF could hardly make any contraversive saccades to remembered or briefly flashed targets if it was initially fixating centrally, but it could make some if it was initially fixating ipsilaterally. The contraversive saccades made from the ipsilateral position were still extremely inaccurate and of long latency (Figure 14). The orbital influence on the monkey's saccadic performance may have been artefactual, arising from the natural centering tendencies of the eye musculature. With the eye initially deviated leftward (Figure 13, top row), weakened FEF signals trying to drive the eye rightward would be aided by the passive muscular tensions.

This idea can also account for the apparent peak velocity depression (see below).

In contrast, the contralaterality of the fixation deficit, another orbital effect, could not have been artefactual. If FEF inactivation caused an equal weakening of fixation at all eye positions, the muscular forces of the eye would have caused equal centering tendencies everywhere. Hence, the fixation deficits would have been as strong for peripheral ipsilateral locations as for contralateral locations, but this was not the case (Figure 16).

Saccadic dynamics: When saccades were generated to briefly flashed targets during FEF inactivation, their peak velocities were significantly decreased from their normal levels in the 2 to 12 degree amplitude range (Figure 19 B and D). Interestingly, this range was similar to the amplitude range of saccades that were electrically evoked from this part of the FEF (5 to 20 degrees, see METHODS).

This effect was somewhat surprising considering that the activity of FEF cells is not correlated with saccadic dynamics (Segraves & Park, 1993). As with the orbital effect, above, it is possible the effect on dynamics was artefactual. The main sequences in the present study had to be derived from initially ipsilateral fixation since only in this condition could any contraversive saccades be generated. Eye movements made from ipsilateral fixation may have been "hybrids", arising from the weakened saccadic signal from the inactive FEF and the normal

centering drifts from the passive muscular forces. The drifts would have lengthened the saccade's amplitude. This would cause the appearance of velocity depression on a main sequence plot.

FEF inactivation vs. SC inactivation

The FEF and the superior colliculus are the two structures through which cortical saccadic signals reach the brainstem (Schiller et al., 1980). It has previously been shown that reversible inactivation of the SC severely disrupts saccades made to long-duration visual targets (Hikosaka & Wurtz, 1985, 1986). The present study found the monkeys with inactivated FEF, in contrast, could make nearly normal contraversive saccades to persistent targets. Therefore, there is a fundamental difference between the FEF and SC: the FEF is much less important than the SC for making saccades to continually present visual stimuli. The FEF is absolutely needed, however, to make saccades to transient visual changes. These results suggest that the evolution of the FEF has improved the monkey's ability to make saccades in more difficult situations, when a peripheral visual stimulus disappears before a saccade can be initiated toward it. The FEF seems to be recruited in the common circumstance of attempting to foveate something that one sees briefly "out of the corner of one's eye".

Conclusion

The two major effects of reversible FEF inactivation are a severe disruption of contraversive saccades and an impairment in the ability to fixate in contralateral space. The appearance of premature ipsiversive saccades using the memory target task and the ipsilateral shift of the eye in darkness are probably due to imbalances between the inactive and normal FEFs. The reasons for the orbital influence on the saccadic deficit, and for the depression of saccadic velocity, are unclear.

At a systems level, we suggest that the FEF is necessary to provide a sustained neural command, E_d, to the brainstem saccadic generator for situations in which the contralateral visual stimulus disappears. At a behavioral level, evolution of the FEF might have improved the ability of the monkey to foveate transient visual changes in the contralateral field.

Legends for Chapter 2

FIGURE 1: Approximate locations of FEF penetrations with respect to the principal sulcus (Ps) and arcuate sulcus (As). The tasks used during lidocaine, muscimol, or saline injection into each of these sites is shown. Sometimes a task and site pair was repeated, which is why the number of tasks shown, 17, is less than the total number of injections, 20 (see text). Position of monkey I's penetrations (right) is known from inspection of sulcal locations during surgery; monkey L's penetrations (left) are estimated from the amplitude topography of FEF (Bruce, Goldberg, Bushnell, & Stanton, 1985).

FIGURE 2: Schematic of the apparatus used to deliver lidocaine, muscimol, or saline into the cortex (not to scale). A hydraulic microdrive system held a microelectrode and needle in parallel and drove both tips through the dura into the brain. Multiple unit activity was monitored from the microelectrode, and the pharmacological agent of choice was infused through the needle.

FIGURE 3: Summary of pilot study undertaken to determine the relationship between lidocaine injection volume and radius of neural inactivation. (A) Typical inactivation data are shown for six tests of a 4 μl injection at 1 mm microelectrode-needle tip separation. Unit activity for each injection is normalized to

its average baseline firing rate (shown at time 0). Neural activity was suppressed on every injection at this volume and distance, and it reached a criterion suppression of 20% of its baseline value for 5 of the 6 injections (exception was the diamond symbols curve). (B) Average of all the inactivation curves in (A) is shown as the 4 μl curve. Average curves for 2 and 7 μl injections, also at 1 mm radius, are shown for comparison. (C) Injections of saline had no effect. (D) Overall relationship of lidocaine injection volume to inactivation radius. Each datum shows the injection volume needed to inactivate units (i.e. drop their firing rate to at least 20% of baseline) at either 1 or 2 mm radius from the needle tip with either 50% (crosses), 70% (asterisks), 90% (diamonds), or 100% (triangles) probability. The dashed lines are linear interpolations between the 1 and 2 mm data. In order to expect neural inactivation at 1.5 mm radius in 100% of injections, an injection volume of at least 18 μl of lidocaine is required (see arrows).

FIGURE 4: In every task used, the monkey initially had 5 seconds to acquire the fixation LED. Once this LED was foveated, the remaining events occurred. (A) Memory target task. 200 msec after fixation, a target LED was lit for 300 msec, then doused. The monkey was required to maintain fixation for 300 msec longer, until the fixation LED turned off, and then make a saccade to the remembered target position. (B) Visual target task. 100 msec

after fixation, the fixation LED disappeared. 100 msec after that, a target LED was lit for either 10, 30, 100, 315, or 1000 msec. The monkey was allowed to go to the target as soon as it appeared. (C) Fixation task. After initial fixation, the monkey was required to maintain its eye position near the LED for 5 seconds.

FIGURE 5: Trajectory deficit during FEF inactivation while the monkey performed the memory target task. Saccadic trajectories superimposed from trials (A) before, (B) during, and (C) after inactivation. Small squares show saccadic endpoints, larger squares represent target locations. Small crosses (mostly obscured) show initial fixation locations. Multiunit firing versus time is shown below, and the periods before, during, and after inactivation corresponding to the data shown in (A), (B), and (C), respectively, are shaded. During the neural inactivation the monkey was severely impaired at making memory saccades to contralateral targets.

FIGURE 6: Saccadic error (left column) and saccadic latency with respect to fixation offset (right column) before, during, and after FEF inactivation for the memory target task. (A) Lidocaine injection (same experiment as in Figure 5), (B) lidocaine injection from the other monkey, (C) muscimol injection, and (D) saline injection. The "during" data are from the first 10 to 30 minutes after lidocaine or saline injection, and from

approximately 90 to 110 minutes after the muscimol injection. "After" data are the last data collected during the lidocaine and saline experiment (usually one to three hours later); for the muscimol experiment, "after" data was collected the following day. One-tailed paired t-tests (df ranged from 5 to 22) were performed to compare data during and after inactivation with data before inactivation, and two different significance levels are shown. The other lidocaine injection (not shown) also had significant error and latency increases during inactivation at the $p < 0.03$ level. Saccades to ipsilateral targets during FEF inactivation and all saccades during saline injection were not significantly changed.

FIGURE 7: Frequency of premature responses to the target flash as a function of time while the monkey performed the memory target task. (A), (B), (C), and (D) correspond respectively to the four experiments of Figure 6. Before the injections, premature saccades occurred as < 20 % of the responses. Just after lidocaine injection, the percentage of all responses that were premature rose markedly, but only for ipsilateral target presentations (dark circles). This effect followed the time-course of neural inactivation (e.g. compare curves in (A) with the neural inactivation plot for this experiment, shown in Figure 5). Muscimol injection (D) caused a later onset of the effect, paralleling the later onset of the effects shown in Figure 6 (D). When there was an obvious difference between results employing

different initial eye positions, the effects were always greater for initially contralateral fixation (thin solid lines) vis-a-vis initially central fixation (dashed lines).

FIGURE 8: Trajectory deficits during FEF inactivation while the monkey performed the visual target task to a 30 msec duration target flash. Conventions as in Figure 5. During the neural inactivation, the monkey was severely impaired at making saccades to contralateral target flashes. The "after" data (C) was collected a few hours after the injection.

FIGURE 9: Time-course of (A) saccadic error and (B) saccadic latency deficits compared with (C) the time-course of FEF neural inactivation, for the experiment shown in Figure 8 (visual target task, 30 msec duration flash). Each datum is the saccadic error (A) or saccadic latency with respect to target onset (B) for a single saccade. Error and latency of saccades made to contralateral targets increase sharply just after the injection, and the neural activity is abolished. As the neural activity recovers, the error and latency of saccades made to contralateral targets also recover. Saccades made to ipsilateral targets are not affected.

FIGURE 10: (A) During FEF inactivation using the visual target task, the first saccade after target onset is often absent or of tiny, < 2 deg., amplitude. This is normally a rare response, as

shown in all the other curves. The effect is greatest for the extremely brief (10 and 30 msec) target flashes (A, "during" data): in such cases, the first response to the target flash is absent or tiny for about 30% of trials.

FIGURE 11: Saccadic error for contralateral (left column) and ipsilateral (right column) target presentations, during FEF inactivation using the visual target task. These data are for those trials in which the first saccade was present and of significant, > 2 deg., amplitude. For lidocaine (A and B) and muscimol (C) injections, saccadic error was significantly elevated for the briefer contralateral targets. Saccades made to long duration (1000 msec) contralateral targets and to all ipsilateral targets were not dysmetric. Saline injection (D) had no effect.

FIGURE 12: Saccadic latency data for the experiments shown in Figure 11. Latency was increased rather uniformly over all target durations for contralateral targets (left column) for the lidocaine (A and B) and muscimol (C) injections. Saline injection (D) had no effect, and saccades made to ipsilateral targets were unchanged (right column).

FIGURE 13: Orbital effect of deficits incurred by FEF inactivation. During the inactivation, some contraversive saccades could be made if the eye was initially placed

ipsilateral to the injection (right column, top). Contraversive saccades were nearly absent, however, if the eye was initially in a central location in the orbit (right column, middle). Same experiment as in Figures 8 and 9.

FIGURE 14: Quantification of orbital effect. (A) Although some contraversive saccades could be made from initially ipsilateral fixation (see Figure 13), they were highly dysmetric and of long latency. (B) A subset of the targets from (A) is selected to match the retinotopic location of targets used in initially central fixation (C). This reveals that the deficits from initially ipsilateral fixation are slightly weaker than those from initially central fixation, all other conditions being equal.

FIGURE 15: Fixation deficits during a FEF inactivation. (A) Thick solid lines represent ipsilateral LEDs, dashed lines central LEDs, and thin solid lines contralateral LEDs. Upper, middle, and lower locations are indicated by triangles, dark squares, and circles, respectively. (B) Acquisition percentage versus time: contralateral LEDs are difficult for the affected monkey to reach, especially the lower right LED. (C) For those trials in which the LED is successfully acquired, the acquisition time to all contralateral LEDs and to the central, lower, LED is increased. (D) Accuracy of fixation once an LED is acquired: the lower and middle contralateral LEDs are very poorly foveated.

Note that all effects follow the normal time-course of neural inactivation (0 to 60 minutes after end of injection).

FIGURE 16: Fixation Deficit Index for all FEF inactivation experiments that used the fixation task. See text for derivation of this index. (A) The lidocaine experiment from Figure 15. Fixation Deficit Index is elevated for all contralateral positions during the FEF inactivation, and especially to the lower one. (B) This lidocaine experiment resulted in little or no effect. (C) This lidocaine experiment resulted in a huge effect, especially to contralateral upper and lower positions, but also to the ipsilateral lower position. (D) Saline injection caused little or no effect.

FIGURE 17: Mean position of the eye in darkness, for all 13 lidocaine injections and the 4 saline injections used to match the lidocaine volume. Before injection, the eye's primary position was always deviated (A) slightly ipsilaterally (leftward) and (B) slightly upward. Just after lidocaine injection (A, thin lines), when the FEF cells shut down, the primary position always shifted even further ipsilaterally, an average of nearly 6 degrees. Saline injections, which never inactivated the FEF, caused no such ipsilateral shift (A, thick lines). (B) There was a slight downward shift in the primary position after saline as well as lidocaine injections.

FIGURE 18: The ipsilateral shift in the eye's primary position in darkness was also seen with muscimol injection. (A) The eye gradually shifted more ipsilaterally over the hours following muscimol injection (thin lines), but not after saline injection (thick line). (B) There was a gradual downward shift after saline as well as muscimol injections.

FIGURE 19: Dynamics of contraversive saccades before and after vs. during FEF inactivation. Peak velocity of each saccade is plotted against its amplitude to form a "main sequence" plot. Left column (A and B): saccades with contraversive component made from ipsilateral fixation using visual target task with 30 msec target duration; pooled data from two experiments. (A) The main sequences before + after vs. during FEF inactivation generally overlap, but there is a predominance of low-amplitude saccades during the inactivation (crosses). (B) Zooming in on the low amplitude range, it is clear that saccades made before and after the inactivation tend to have higher peak velocities than those made during the inactivation. Right column (C and D): saccades with a contraversive component made from ipsilateral fixation using the memory target task. (C) Again, there is a general overlap of the main sequences, but (D) the saccades made before and after FEF inactivation tend to have higher peak velocities than those made during the inactivation, for the 2 to 12 degree amplitude range.

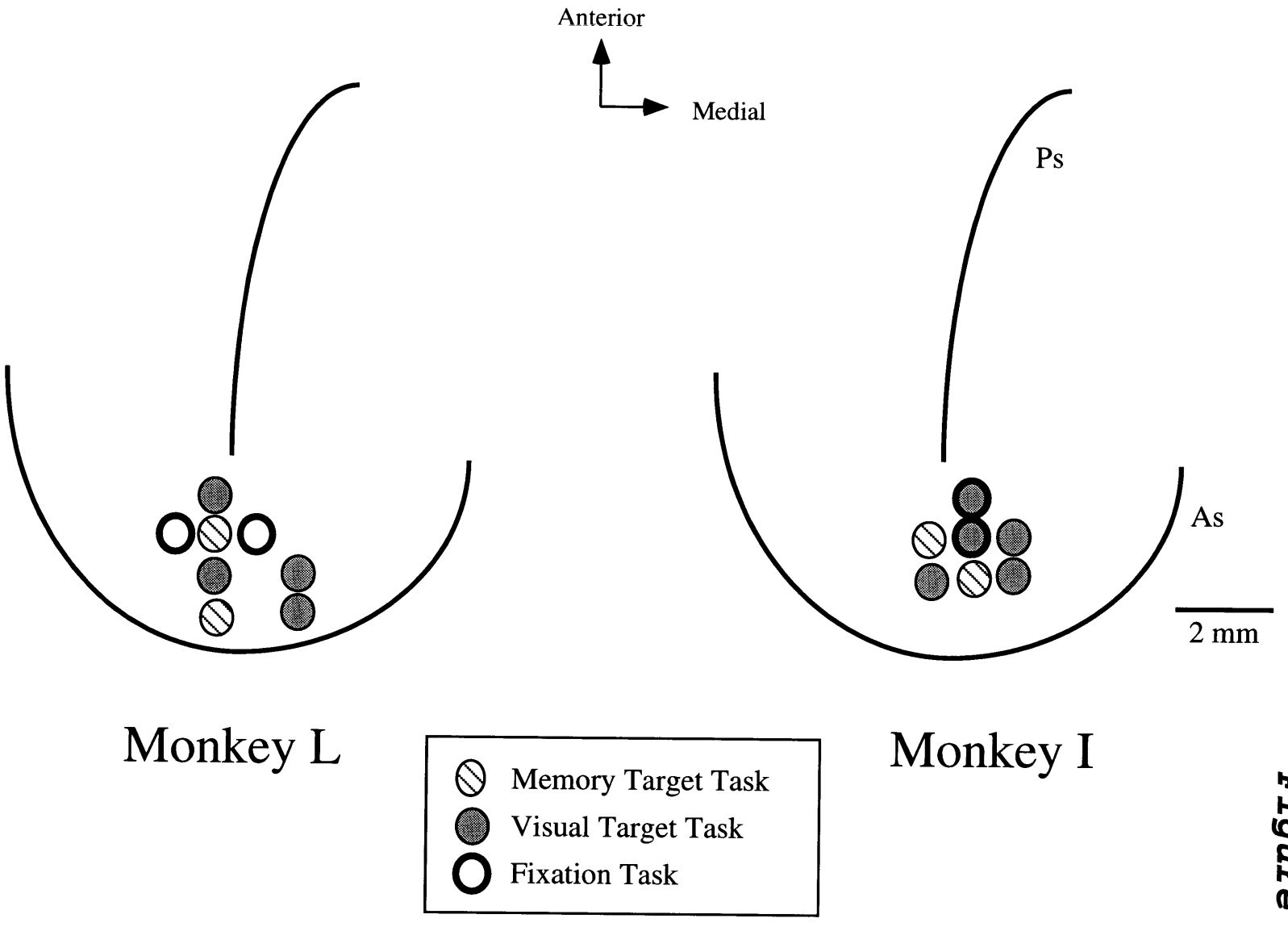

Anterior
Medial
Ps
As
2 mm
Monkey L
Monkey I
Memory Target Task
Visual Target Task
Fixation Task

Figure 1

Figure 2

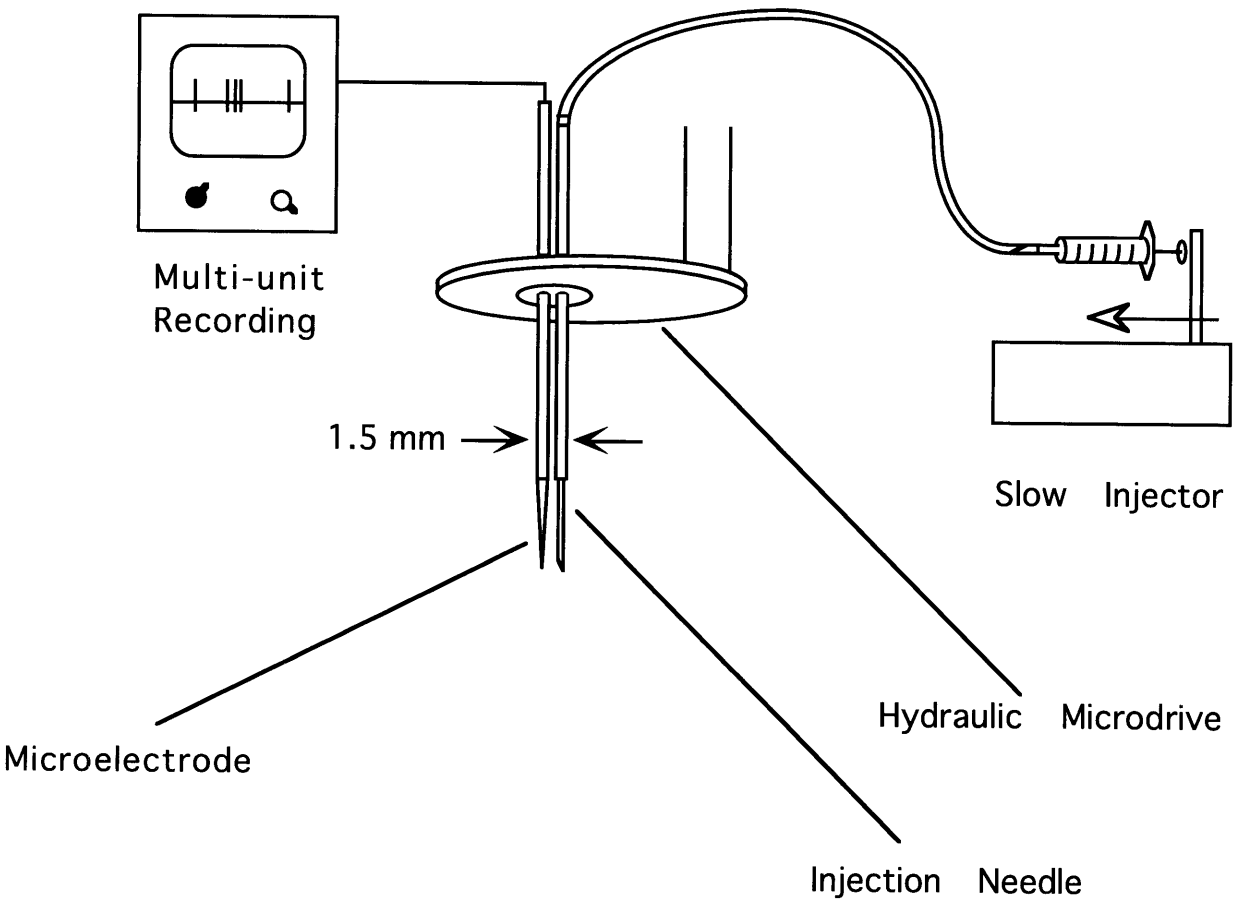

Multi-unit
Recording
1.5 mm
Slow Injector
Hydraulic Microdrive
Microelectrode
Injection Needle

Figure 3

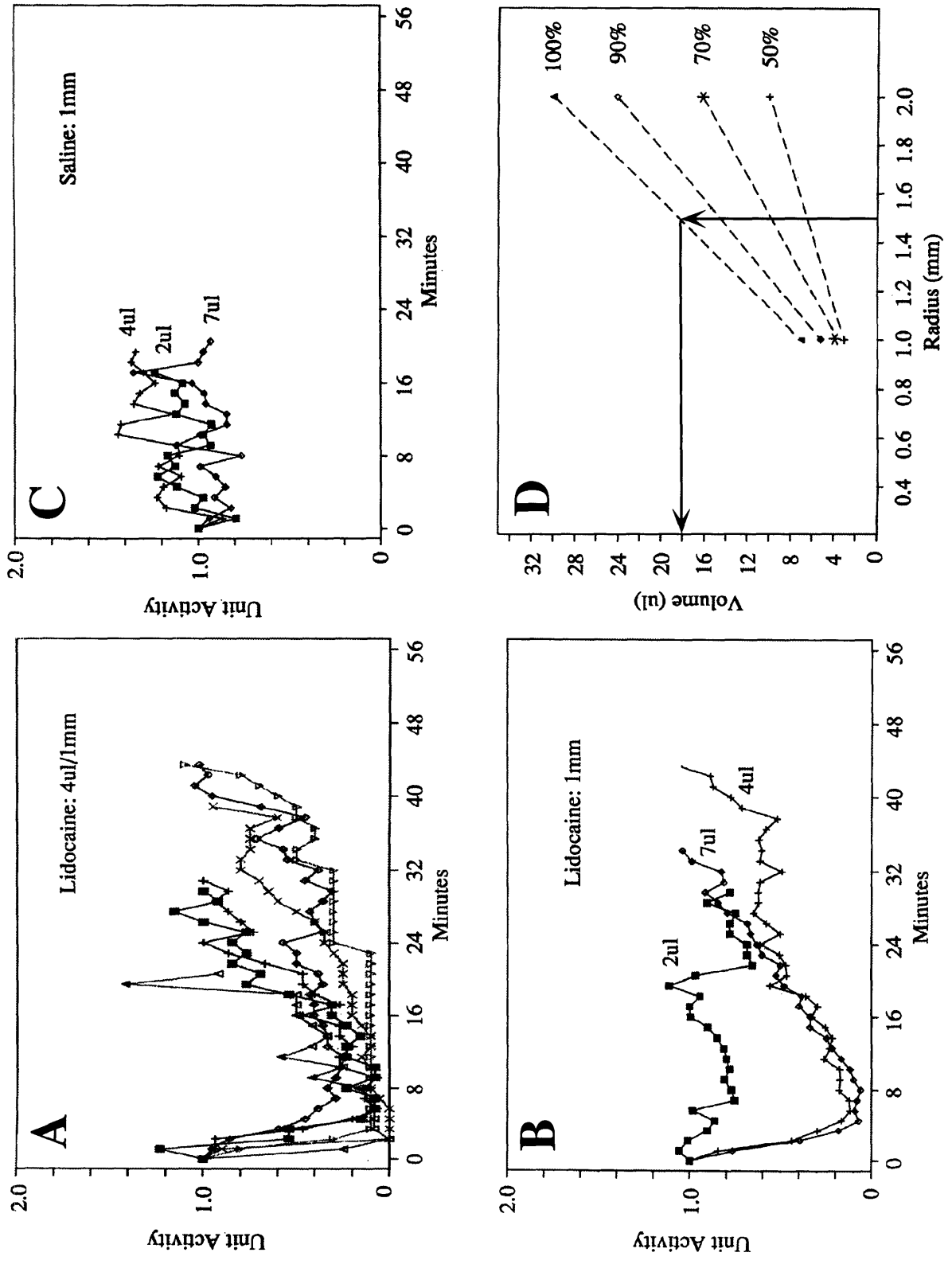
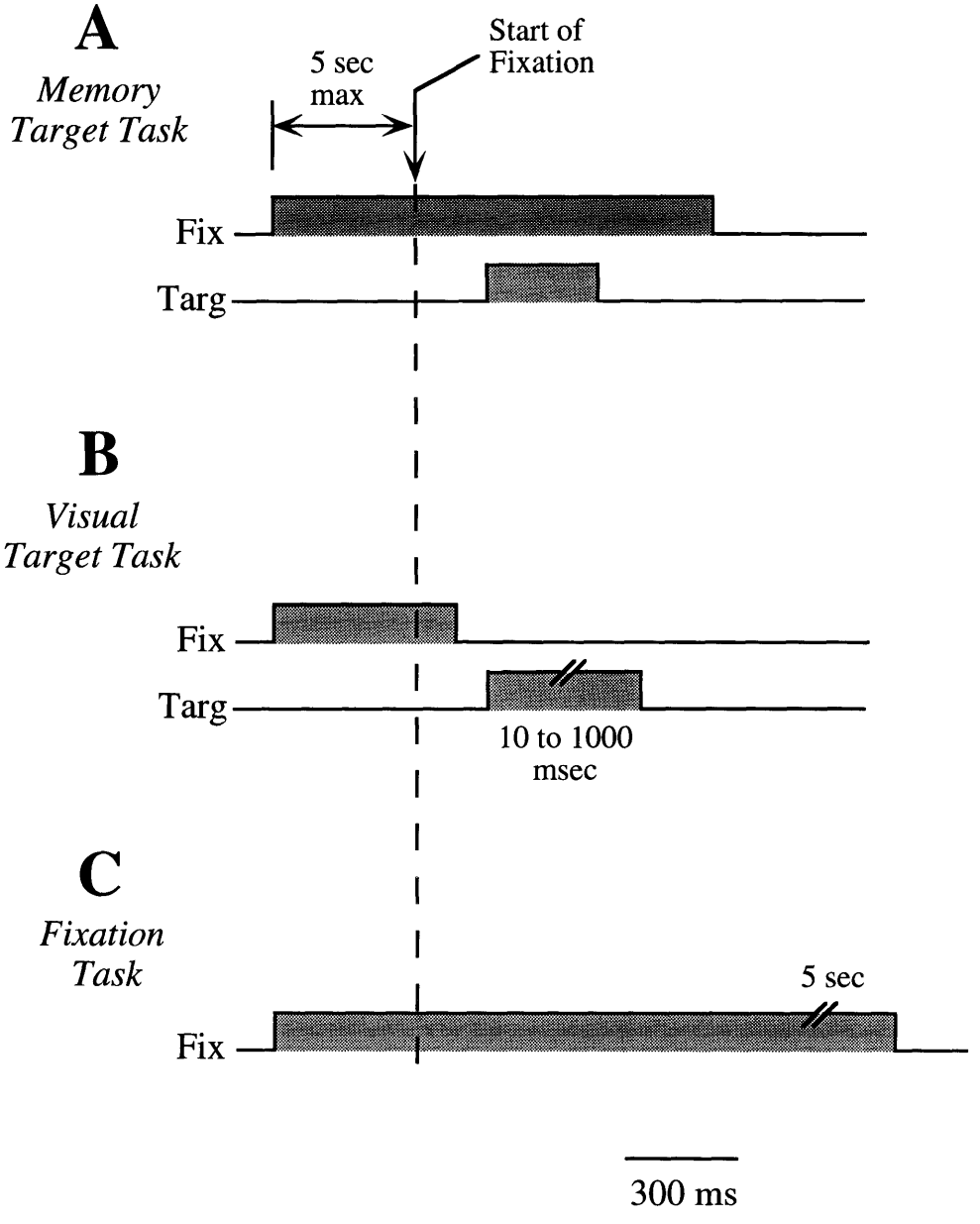

A
Lidocaine: 4ul/1mm
Unit Activity
Minutes
B
Lidocaine: 1mm
2ul
7ul
4ul
C
Saline: 1mm
4ul
2ul
7ul
D
100%
90%
70%
50%
Volume (ul)
Radius (mm)

Figure 4


A
Memory
Target Task
5 sec
max
Start of
Fixation
Fix
Targ
B
Visual
Target Task
Fix
Targ
10 to 1000
msec
C
Fixation
Task
5 sec
Fix
300 ms

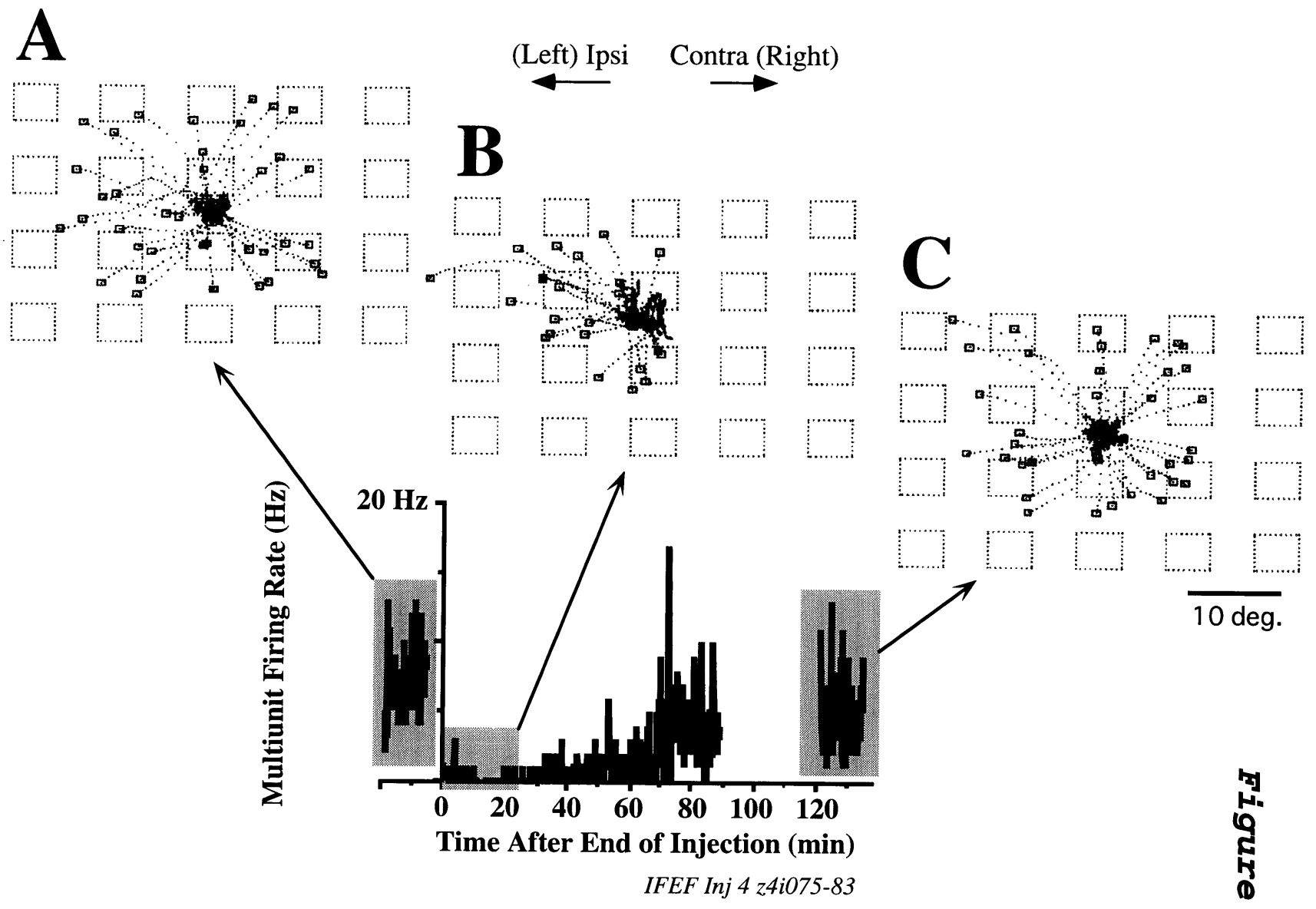

A
(Left) Ipsi
Contra (Right)
B
C
20 Hz
Multiunit Firing Rate (Hz)
10 deg.
0
20
40
60
80
100
120
Time After End of Injection (min)
IFEF Inj 4 z4i075-83

Figure 5

Figure 6

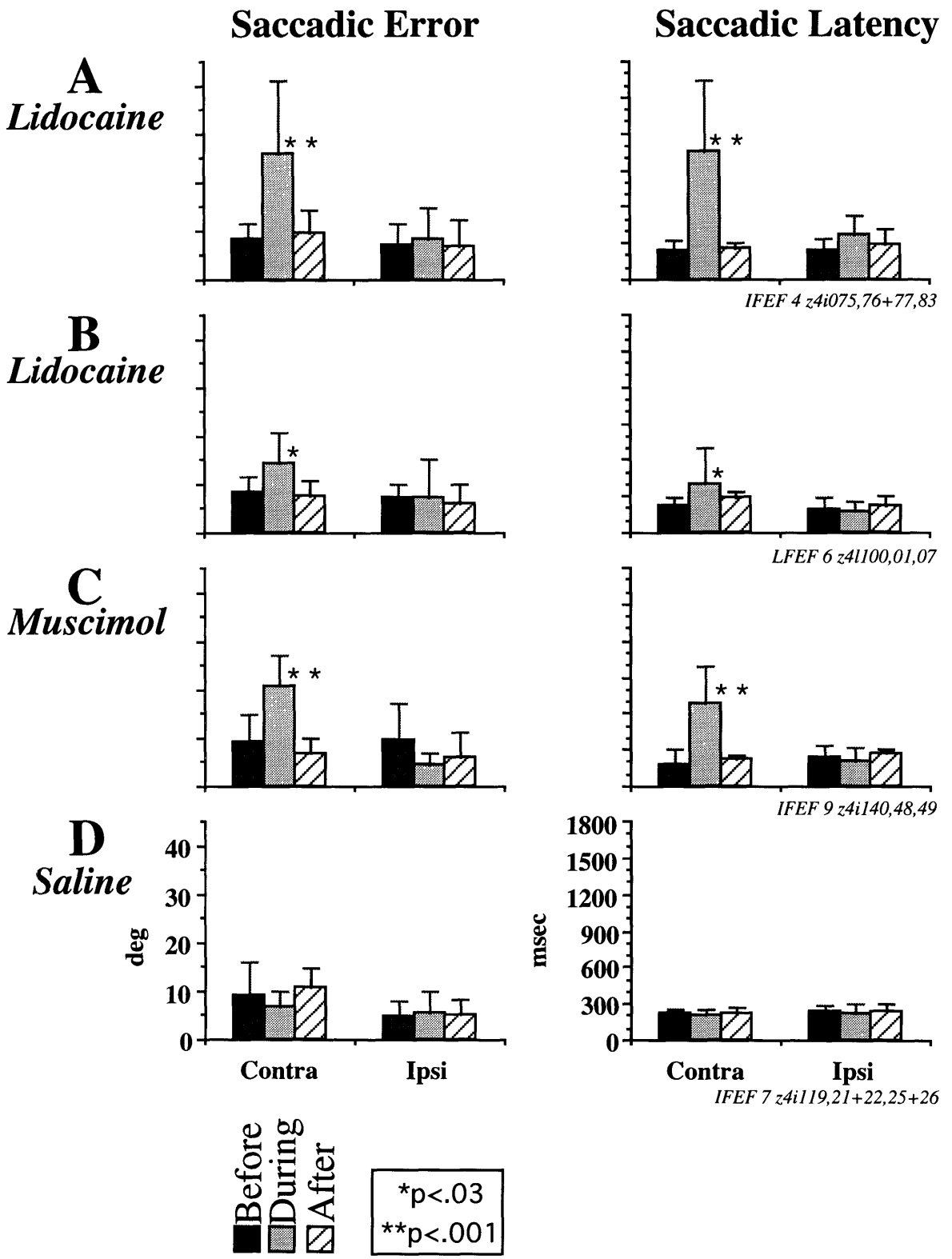
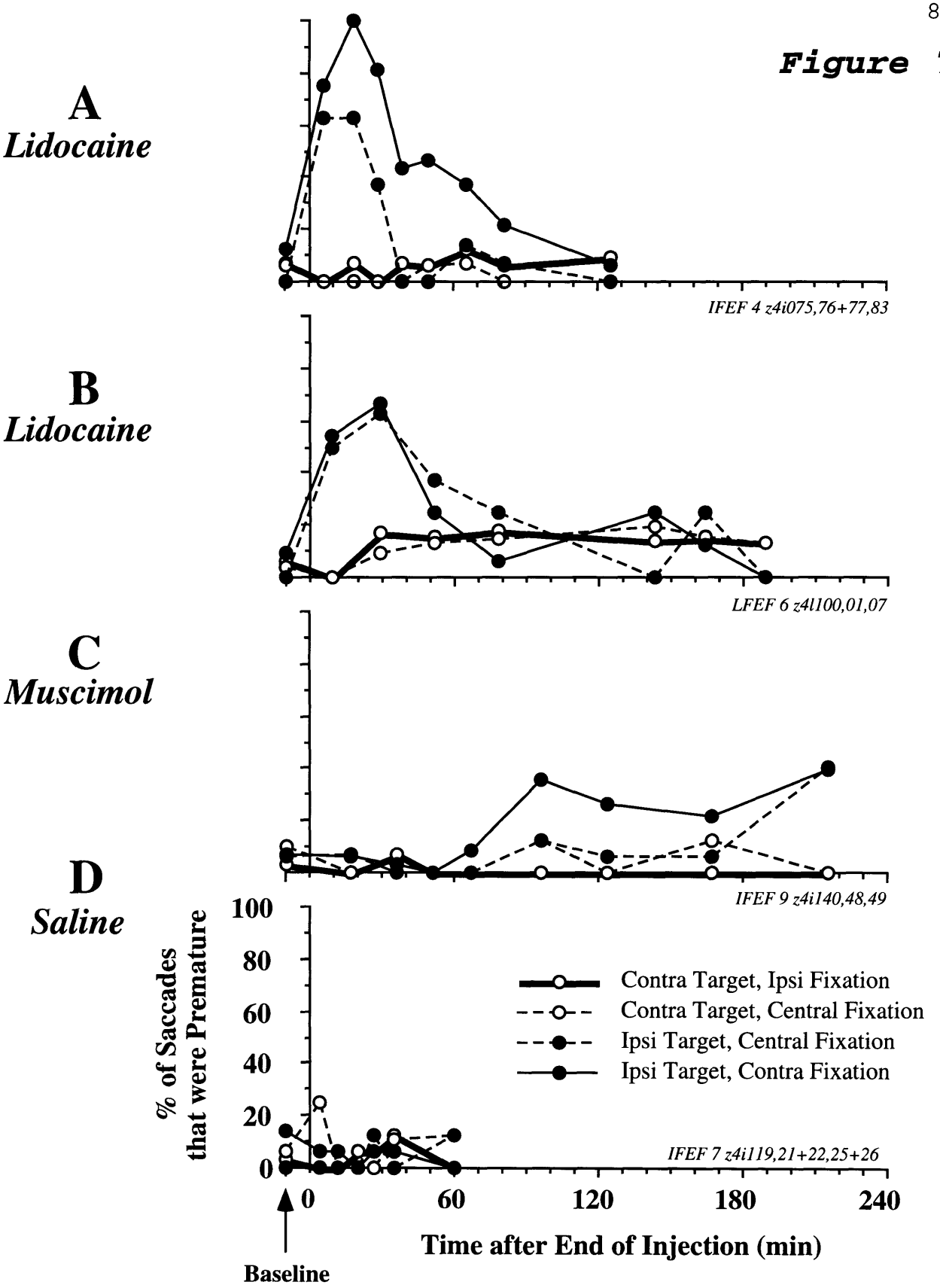

Saccadic Error
Saccadic Latency
A
Lidocaine
IFEF 4 z4i075,76+77,83
B
Lidocaine
LFEF 6 z4l100,01,07
C
Muscimol
IFEF 9 z4i140,48,49
D
Saline
deg
msec
40
30
20
10
0
1800
1500
1200
900
600
300
0
Contra
Ipsi
IFEF 7 z4i119,21+22,25+26
Before
During
After
*p<.03
**p<.001

Figure 7


A
Lidocaine
IFEF 4 z4i075,76+77,83
B
Lidocaine
LFEF 6 z4l100,01,07
C
Muscimol
IFEF 9 z4i140,48,49
D
Saline
% of Saccades that were Premature
100
80
60
40
20
0
Contra Target, Ipsi Fixation
Contra Target, Central Fixation
Ipsi Target, Central Fixation
Ipsi Target, Contra Fixation
IFEF 7 z4i119,21+22,25+26
0
60
120
180
240
Time after End of Injection (min)
Baseline

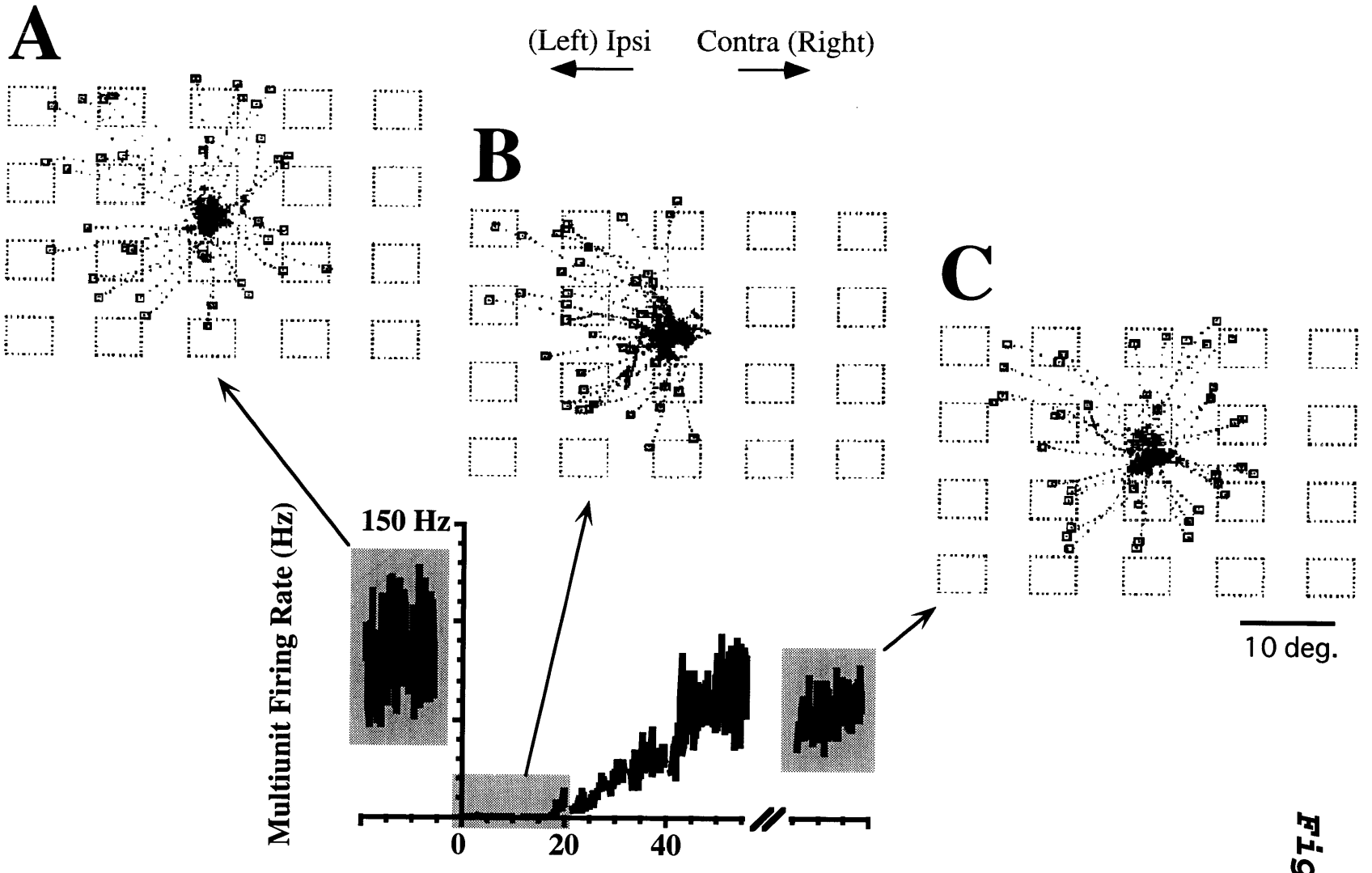
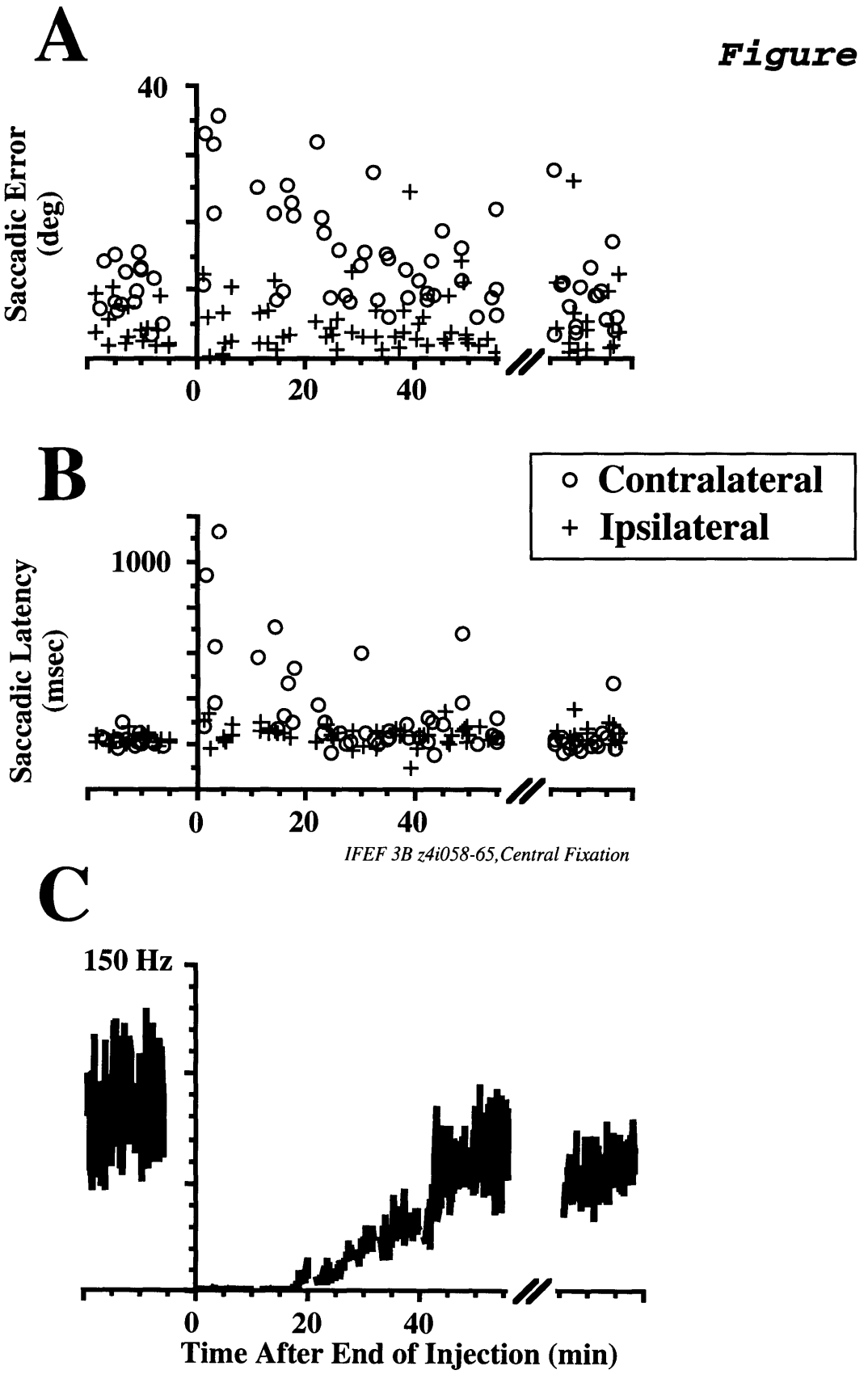

A
(Left) Ipsi
Contra (Right)
B
C
150 Hz
Multiunit Firing Rate (Hz)
0
20
40
Time After End of Injection (min)
IFEF Inj 3B z4i058-65
10 deg.

Figure 8

Figure 9


A
40
Saccadic Error (deg)
0
20
40
B
Contralateral
Ipsilateral
1000
Saccadic Latency (msec)
0
20
40
IFEF 3B z4i058-65,Central Fixation
C
150 Hz
0
20
40
Time After End of Injection (min)

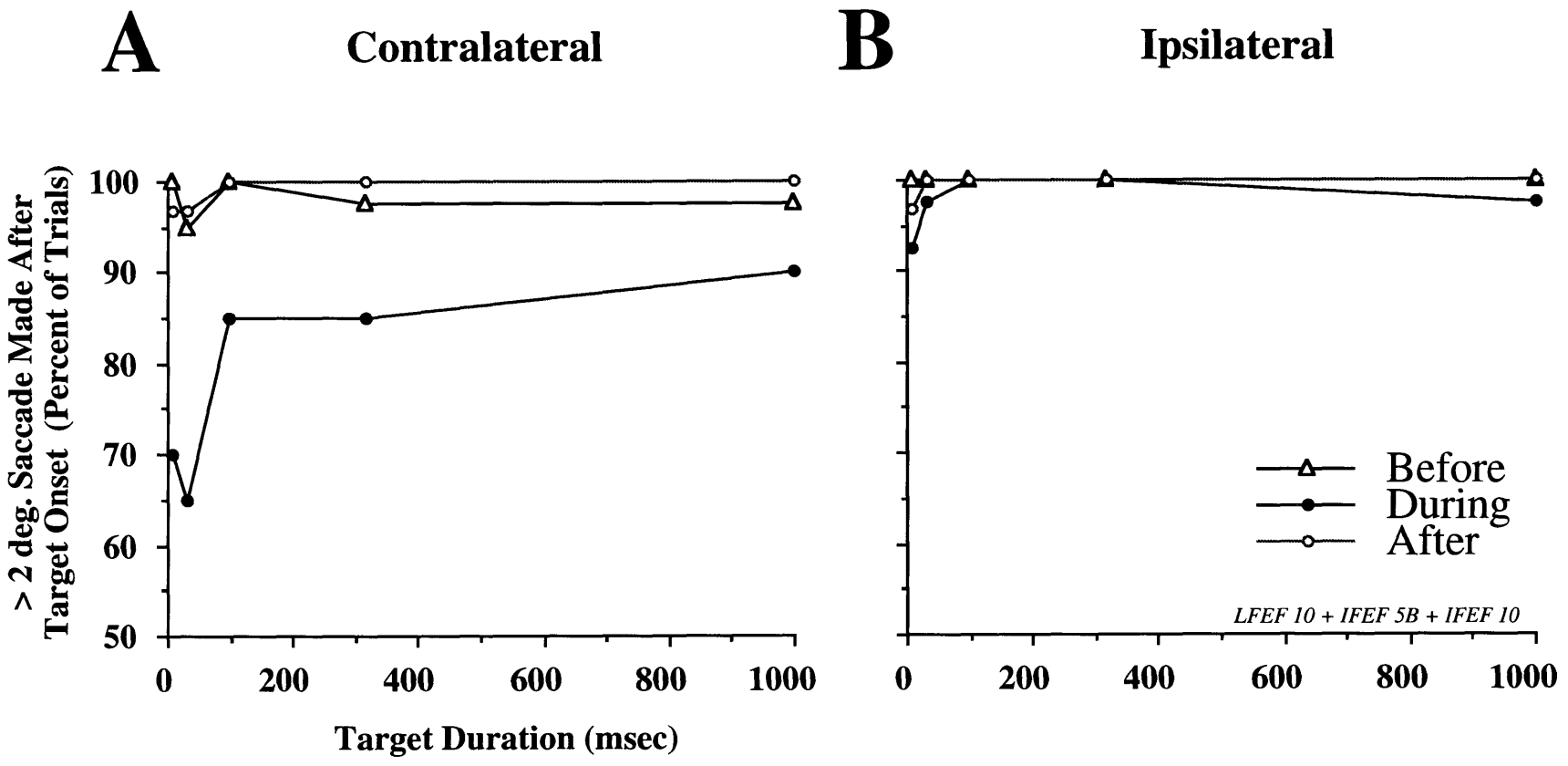

A
Contralateral
B
Ipsilateral
> 2 deg. Saccade Made After Target Onset (Percent of Trials)
100
90
80
70
60
50
0
200
400
600
800
1000
Target Duration (msec)
Before
During
After
LFEF 10 + IFEF 5B + IFEF 10

Figure 10

Figure 11

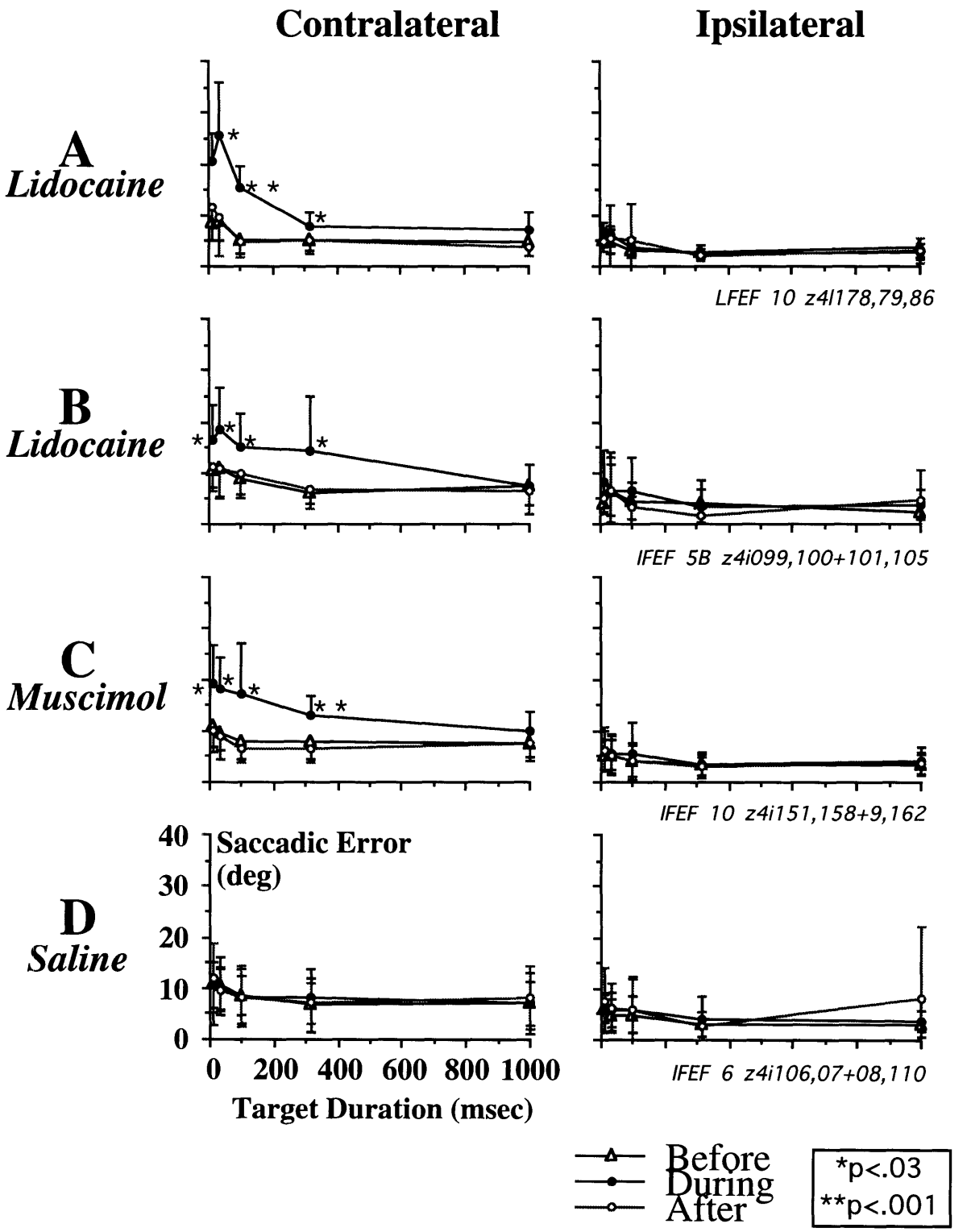

Contralateral
Ipsilateral
A
Lidocaine
LFEF 10 z4l178,79,86
B
Lidocaine
IFEF 5B z4i099,100+101,105
C
Muscimol
IFEF 10 z4i151,158+9,162
D
Saline
Saccadic Error (deg)
40
30
20
10
0
0 200 400 600 800 1000
Target Duration (msec)
IFEF 6 z4i106,07+08,110
Before
During
After
*p<.03
**p<.001

Figure 12

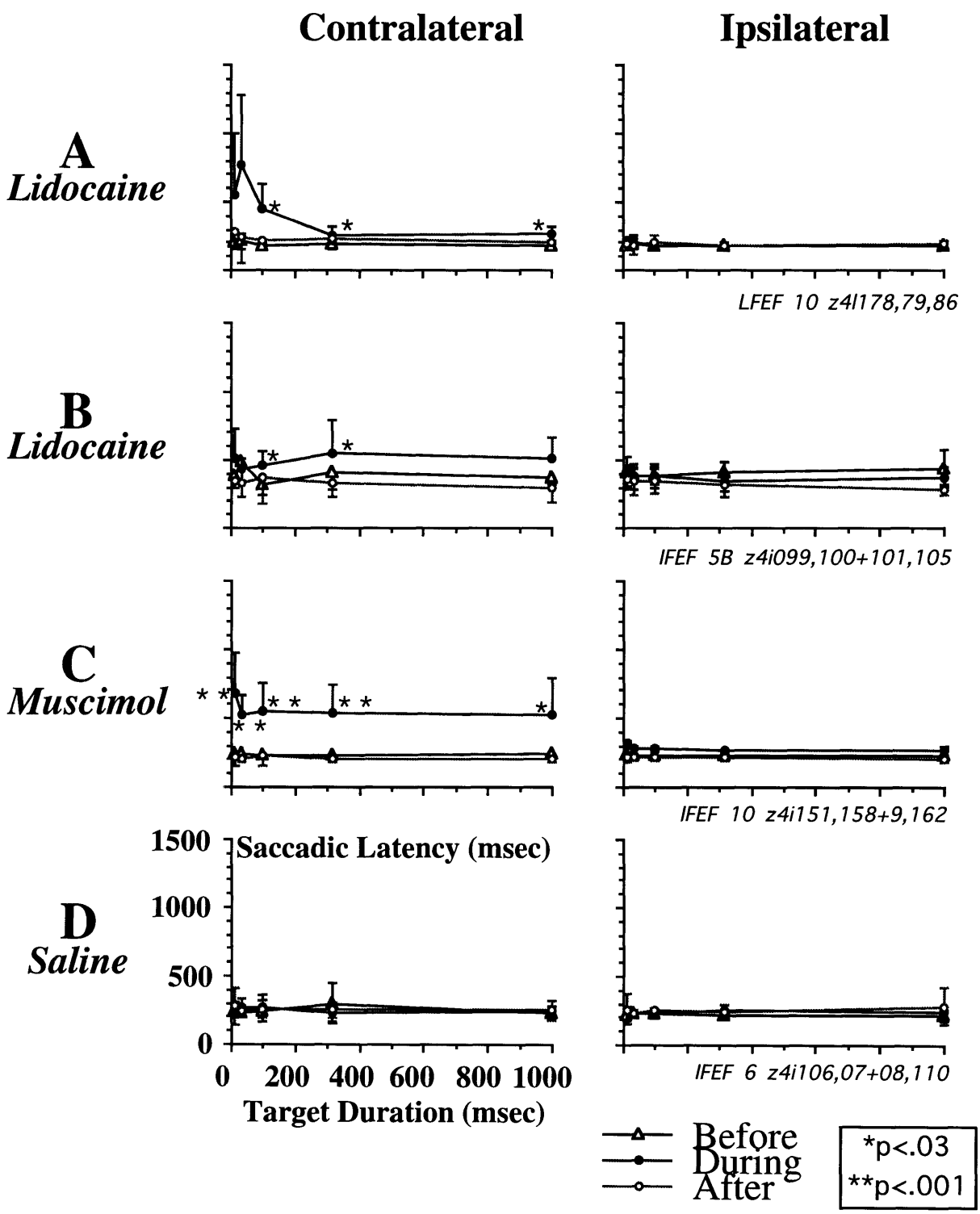
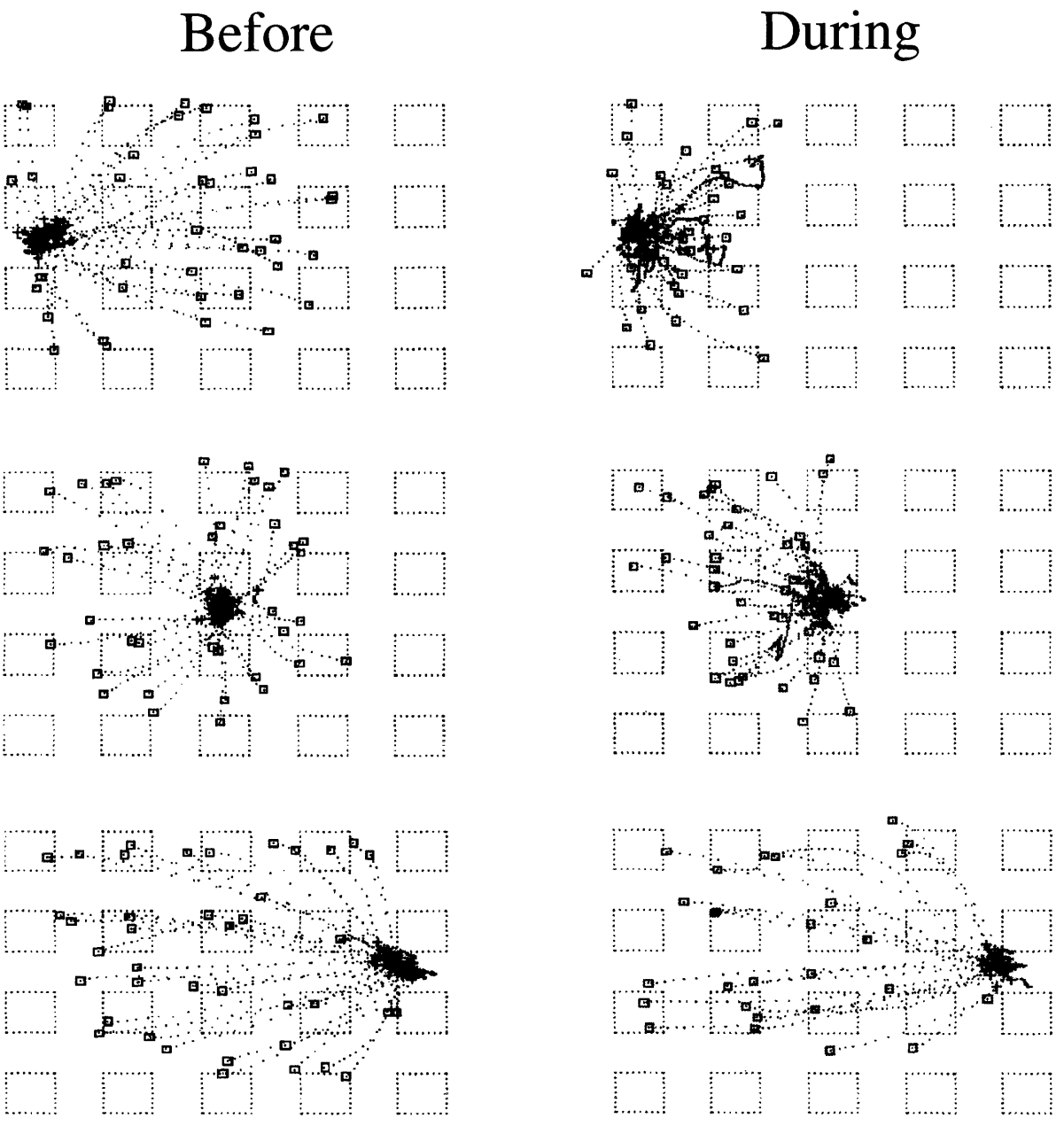

Contralateral
Ipsilateral
A
Lidocaine
LFEF 10 z4l178,79,86
B
Lidocaine
IFEF 5B z4i099,100+101,105
C
Muscimol
IFEF 10 z4i151,158+9,162
D
Saline
Saccadic Latency (msec)
1500
1000
500
0
0 200 400 600 800 1000
Target Duration (msec)
IFEF 6 z4i106,07+08,110
Before
During
After
*p<.03
**p<.001

Figure 13



IFEF Inj 3B z4i058,59+60

Figure 14

A
B
C
30 deg.
40 deg.
Fix
Targets
Saccadic Latency (msec)
Saccadic Error (deg)
Before
During
After
*p<.03
**p<.001
IFEF 3B z4i058,59+60,65

Figure 15

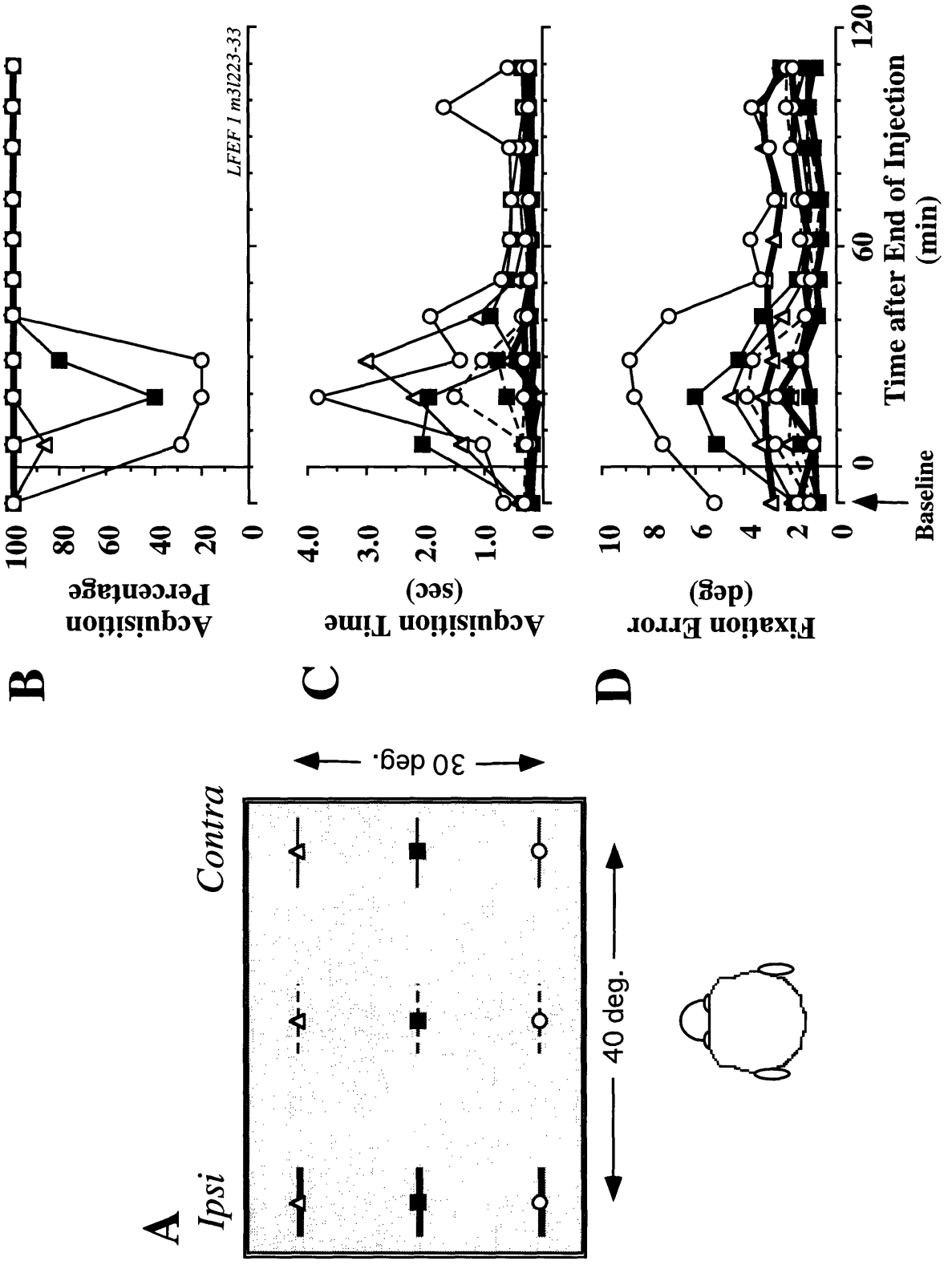

A
Ipsi
Contra
30 deg.
40 deg.
B
Acquisition Percentage
100
80
60
40
20
0
LFEF 1 m3l223-33
C
Acquisition Time (sec)
4.0
3.0
2.0
1.0
0
D
Fixation Error (deg)
10
8
6
4
2
0
Baseline
0
60
120
Time after End of Injection (min)

Figure 16

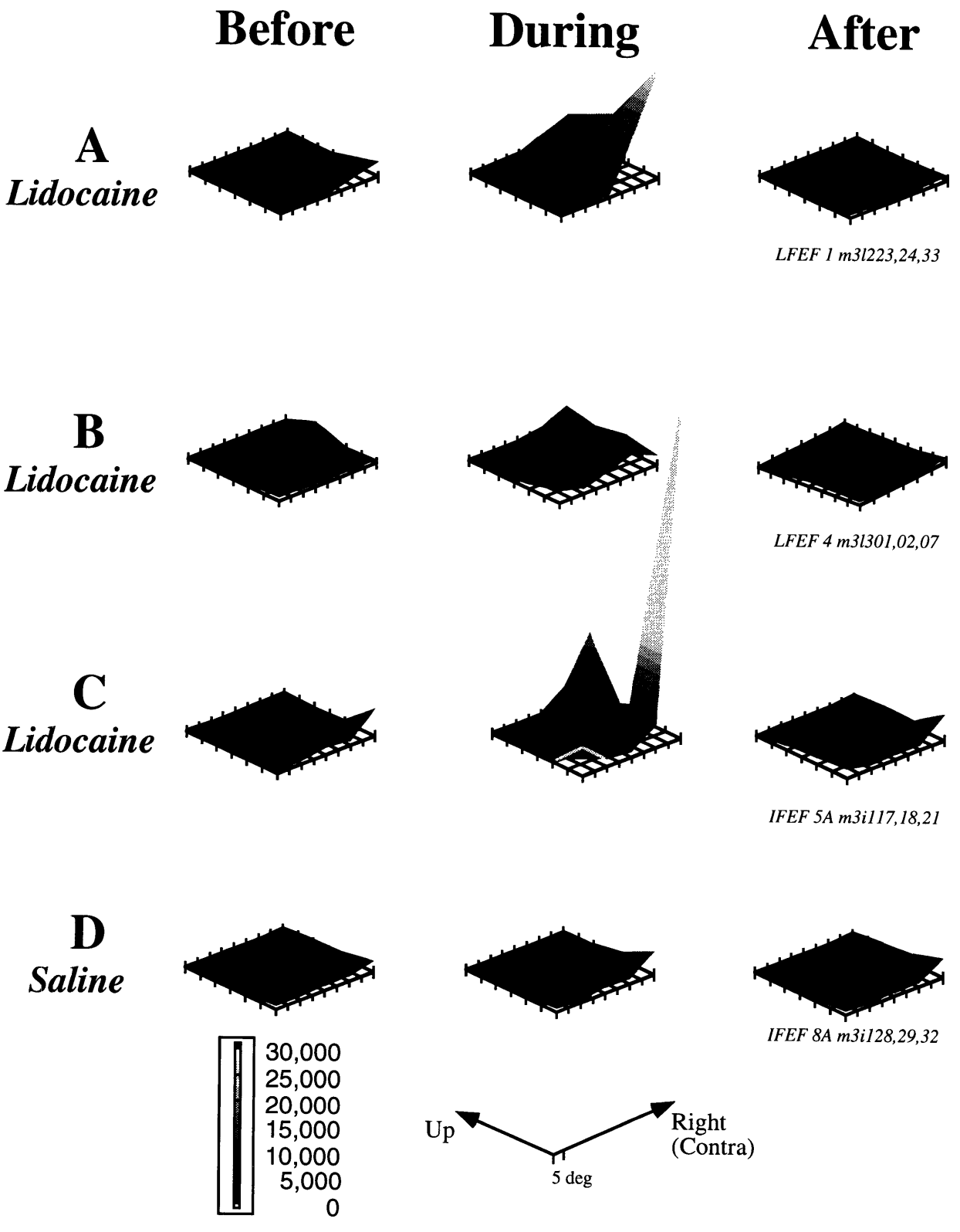

Before
During
After
A
Lidocaine
LFEF 1 m3l223,24,33
B
Lidocaine
LFEF 4 m3l301,02,07
C
Lidocaine
IFEF 5A m3i117,18,21
D
Saline
IFEF 8A m3i128,29,32
30,000
25,000
20,000
15,000
10,000
5,000
0
Up
Right
(Contra)
5 deg

Figure 17

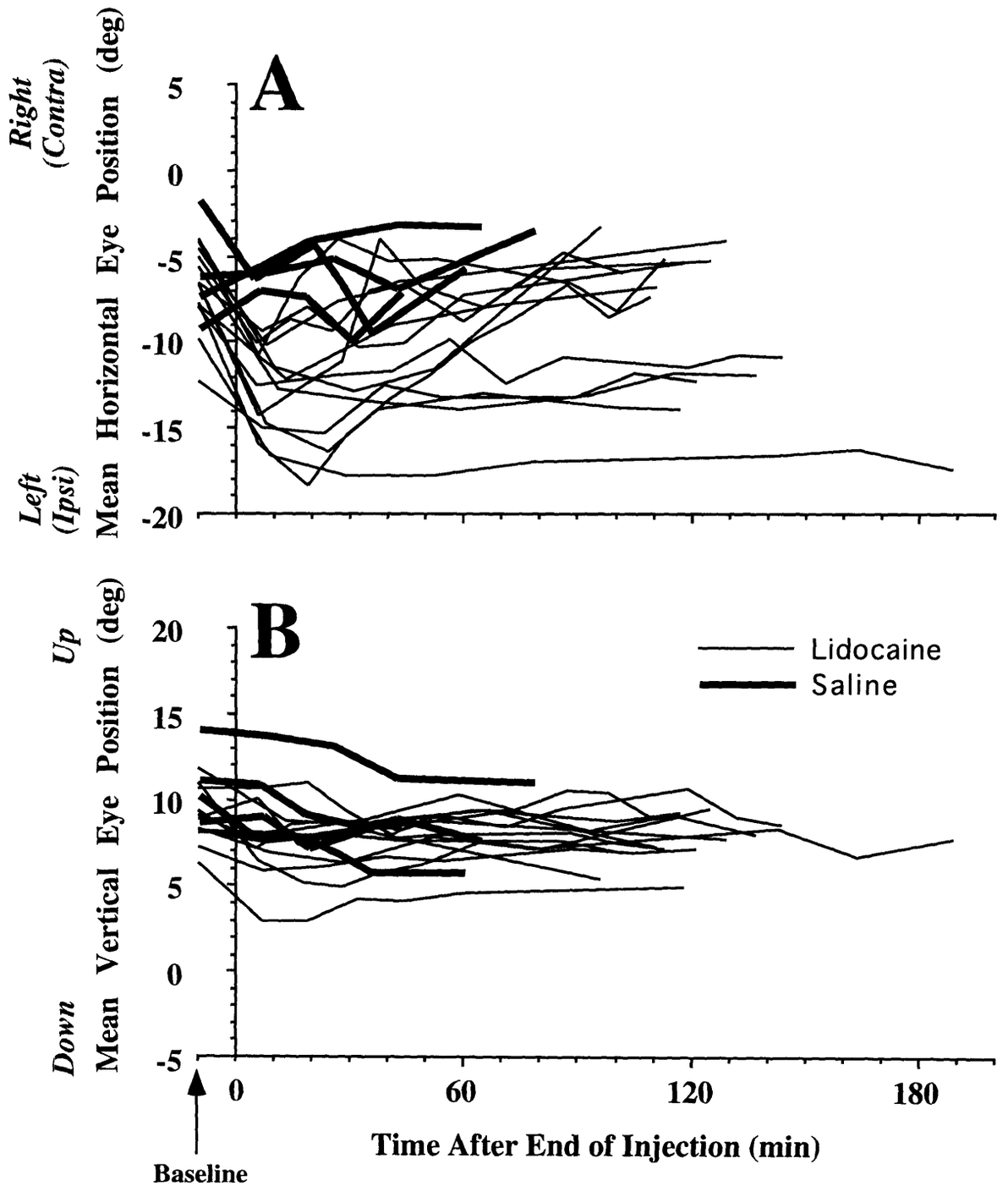
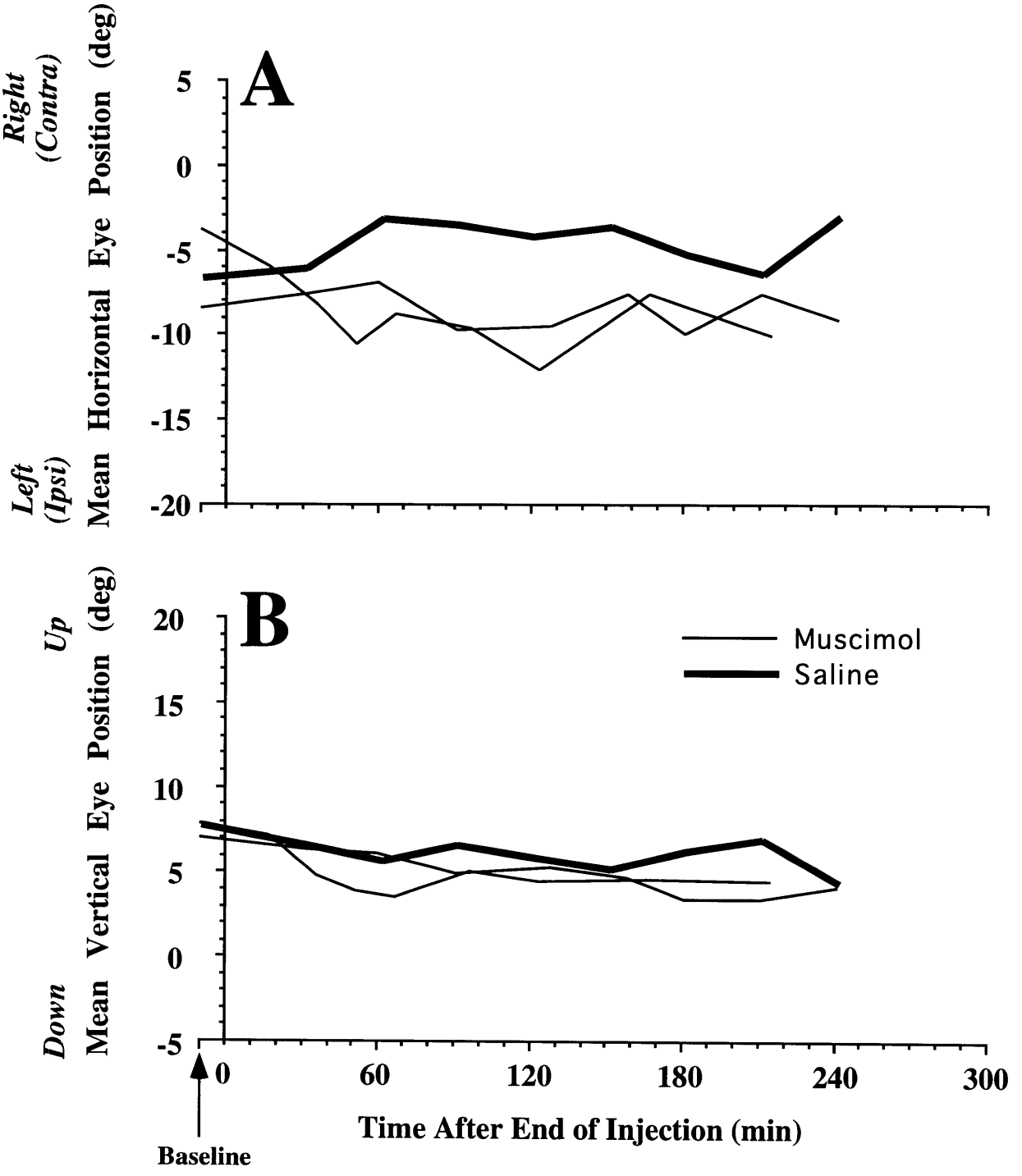

A
Right (Contra)
Left (Ipsi)
Mean Horizontal Eye Position (deg)
5
0
-5
-10
-15
-20
B
Up
Down
Mean Vertical Eye Position (deg)
20
15
10
5
0
-5
Lidocaine
Saline
0
60
120
180
Time After End of Injection (min)
Baseline

Figure 18


A
Right (Contra)
Left (Ipsi)
Mean Horizontal Eye Position (deg)
5
0
-5
-10
-15
-20
B
Up
Down
Mean Vertical Eye Position (deg)
20
15
10
5
0
-5
Muscimol
Saline
0
60
120
180
240
300
Time After End of Injection (min)
Baseline

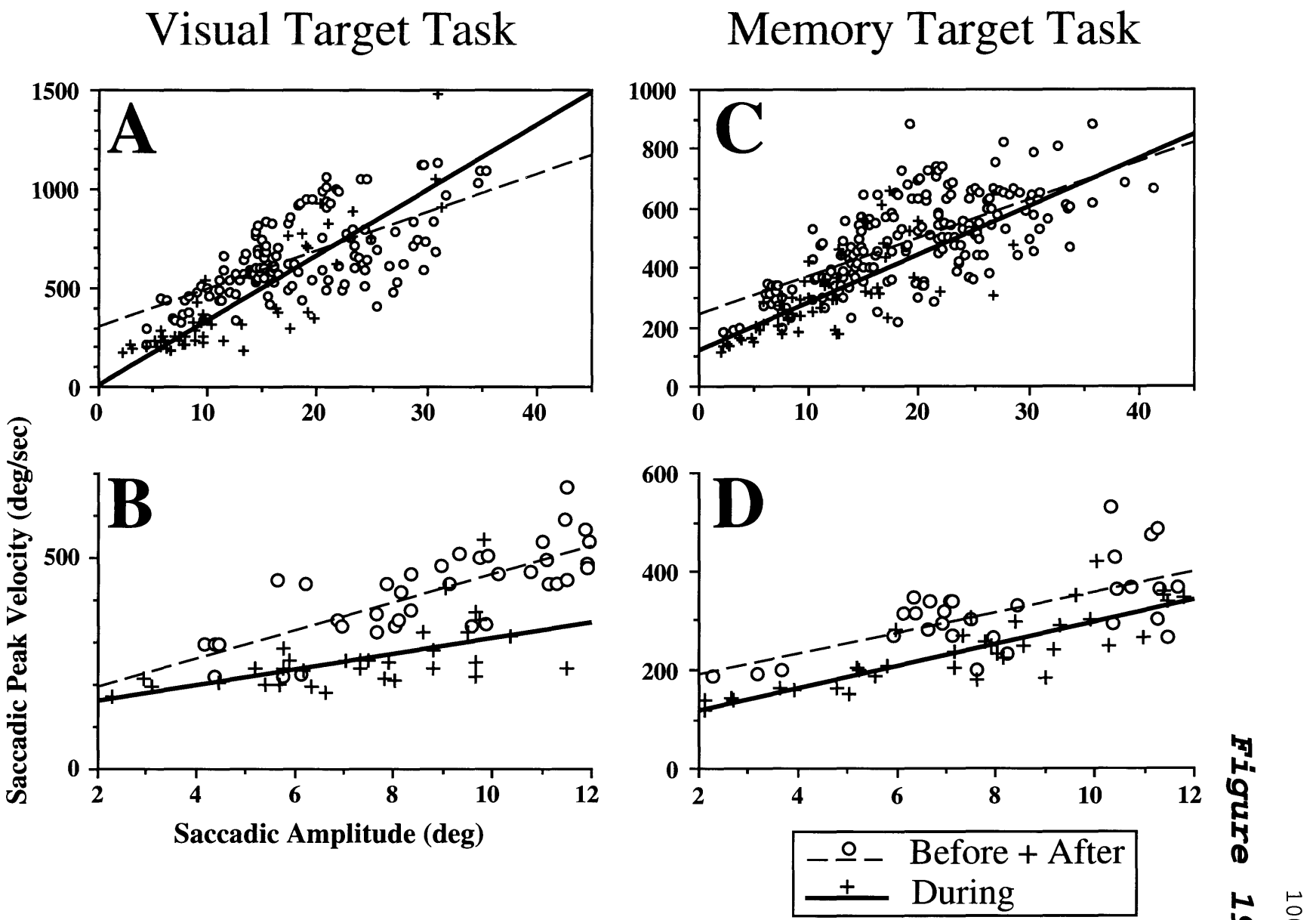

Visual Target Task
Memory Target Task
A
B
C
D
Saccadic Peak Velocity (deg/sec)
Saccadic Amplitude (deg)
Before + After
During

Figure 19

Chapter 3

Reversible Inactivation of the Dorsomedial Frontal Cortex

Introduction

The respective functions of the dorsomedial frontal cortex (DMFC) and the frontal eye fields (FEFs) in the generation of saccades and fixations are unknown. Controversy exists in the study of these two areas, since some authors find them to be uninterestingly similar and some find that they are profoundly different.

There seem to be many ways in which the DMFC and FEF differ. The DMFC contains cells that not only fire in association with eye movements but also with reaching forelimb movements (Brinkman & Porter, 1979; Schall, 1991a; Mann, Thau, & Schiller, 1988). Electrical stimulation of DMFC elicits body movements as well as eye movements (Schlag & Schlag-Rey, 1987; Mitz & Wise, 1987; Tehovnik & Lee, 1993). Both contra- and ipsiversive saccadic eye movements, as well as fixations, can be evoked from stimulation of DMFC (Schlag & Schlag-Rey, 1987; Tehovnik & Lee, 1993). DMFC stimulation causes saccades that converge at a position in space (Schlag & Schlag-Rey, 1987; Mann et al., 1988; Mitz & Godschalk, 1989; Schall, 1991a; Bon & Lucchetti, 1992; Tehovnik & Lee, 1993). Lesion studies confirm that the DMFC is less involved in the retinotopic encoding of saccades than with the spatial relationship of saccades and targets (Pierrot-Deseilligny, Israel, Berthoz, Rivaud, & Gaymard, 1993; Gaymard, Pierrot-Deseilligny, & Rivaud, 1990). Single-unit recording studies have

emphasized basic oculomotor differences between the two areas (Schlag & Schlag-Rey, 1987; Mann et al. 1988; Lee & Tehovnik, 1995). In addition, a recent recording study (Chen & Wise, 1995a,b) demonstrated that DMFC cells, more than FEF cells, modulate their firing during learning of visuomotor associations. In summary, it seems that the DMFC might be involved in higher level functions than the FEF.

Other authors note the common relationships between the two areas: 1) the DMFC and FEF are highly interconnected (Huerta, Krubitzer, & Kaas, 1987; Huerta & Kaas, 1990; Schall, Morel, & Kaas, 1993); 2) the properties of saccade-related cells (Schall, 1991b; Russo & Bruce, 1991) and fixation-related cells (Bon & Lucchetti, 1992) in the DMFC may be similar to those in FEF; 3) one group insists that stimulation-evoked saccades from the two areas have identical characteristics (Russo & Bruce, 1993); and 4) it is clear that both the FEF and the DMFC project to similar cortical and subcortical structures (FEF: Huerta et al., 1986, 1987; Stanton, Goldberg, & Bruce, 1988a,b; DMFC: Huerta & Kaas, 1990; Shook, Schlag-Rey, & Schlag, 1988, 1990, 1991).

In the accompanying report we demonstrated that reversible inactivation of the FEF causes deficits in the generation of saccades and fixation. Now we ask the question: How does reversible inactivation of the DMFC compare with inactivation of the FEF? In this report we describe the oculomotor effects of reversible DMFC inactivation when the monkey performs two classes of tasks: the simple, single-saccade tasks that were used in the

FEF study, and a more complex double-saccade task that might be even more likely to necessitate involvement of the DMFC.

It should be emphasized that it is logically necessary that the simpler, single-saccade tasks be tested first during DMFC inactivation before trying higher level tasks. This is true even though the literature suggests that deficits are likely in motor sequencing, learning, or memory tasks during DMFC inactivation. It would, of course, be intellectually thrilling to immediately try such higher level tasks. But deficits seen in the performance of a complex task like the double-saccade task are better interpreted if the deficits to the task's component actions, i.e. two successive single saccades, are already fully understood. We believe this rigorous, reductionist approach is a sound first step in approaching a daunting and controversial area such as the DMFC.

We found that reversible DMFC inactivation caused neither saccadic nor fixational deficits in the single-saccade tasks, in marked contrast to the effects of reversible FEF inactivation. However, when the DMFC was inactivated, the monkey's ability to plan and execute two saccades in sequence was impaired. This was an omnidirectional deficit, i.e. it was mostly irrespective of target position in space. In contrast, the FEF is necessary for the proper execution of both single- and double-saccade tasks, and inactivation of the FEF always causes highly contralateralized deficits. These results support the position that there are marked differences between the FEF and DMFC, both

in basic function and in laterality of influence.

Methods

Monkeys

The same monkeys, designated as monkey L and monkey I, were used in this study as in the accompanying FEF study. We will only briefly describe the preparation of these animals for this study. Implantation of a head post and an eye coil was accomplished during an initial surgery. After a monkey was sufficiently trained on simple fixation and eye movement tasks, a second surgery was performed to implant a chamber over the DMFC. In monkey L, the DMFC chamber was centered on the midline at AP coordinate +27.5. In monkey I, the DMFC chamber was placed at AP +25 and was intentionally centered 3 mm to the right of the midline, to leave adequate room for the left FEF chamber in this monkey. A sketch of the location of the superior saggital sulcus, visible through the dura, was made to help guide the subsequent stimulation mapping. Monkeys were deprived of water overnight before testing, in accordance with NIH and MIT guidelines.

Identification of DMFC locations with stimulation mapping and unit properties

We mapped out the location of the DMFCs in the chambers of each monkey using the techniques described in the FEF study. Briefly, in each penetration we electrically stimulated from the first unit encountered, down until no more units were encountered. The stimulation parameters were the same as were used in the FEF, except for a longer train duration: biphasic pulses, 0.1 msec pulse duration, 150 Hz, 800 msec train duration. The longer train duration was necessary because of the long latency of evoked saccades whenever the initial eye position was near the termination zone of a site (Tehovnik & Lee, 1993). In contrast to the FEF, these long durations rarely evoked staircase saccades; rather, they almost always evoked a single saccade followed by a fixation in the termination zone.

Saccades or fixations could be evoked throughout a DMFC penetration, from the first unit encountered to the last unit. In both monkeys we found the topographic layout of termination zones as described by Tehovnik & Lee (1993). Examples of stimulation-evoked saccades are illustrated in Figure 1. The cortical depth of effective stimulation ranged from the surface down 4 mm deep in the lateral DMFC and down to > 8 mm deep in the medial DMFC; the electrode was going down the bank of the superior saggital sulcus in the medial DMFC. This knowledge was methodologically important because it allowed for logical needle

tip placement for injections (see below).

Unit recording verified that we were in the DMFC eye field. The right DMFC of monkey L had been extensively studied by Lee & Tehovnik (1995) in a single-unit study. Furthermore, the area from which we evoked saccades coincided with the dorsomedial eye field as defined with single-unit and electrical stimulation studies of other authors (Schlag & Schlag-Rey, 1987; Schall, 1991a; Bon & Lucchetti, 1992; see Tehovnik, 1995, for review). Finally, with both monkeys throughout the present study, whenever we were recording from multiple or single units we commonly encountered activity related to saccadic eye movements, fixations, vision, or some combination of these. Hence, we are confident from both the stimulation and unit properties that we were in the DMFC eye field.

The electrical stimulation current thresholds of this area were < 400 μA, using our parameters. The current thresholds ranged from 50 to 100 μA using the stimulation parameters of Russo & Bruce (1993). Therefore we were probably caudal and medial to the region of highest electrical sensitivity, termed the supplementary eye field (SEF) (Russo & Bruce, 1993; Matelli, Luppino, & Rizzolatti, 1991; Luppino, Matelli, Camarda, Gallese, & Rizzolatti, 1991; see Tehovnik, 1995 for review). It is certainly possible that inactivation of the highly-sensitive SEF might give different results from those presented here for DMFC inactivation.

Alternation of FEF and DMFC injections

For monkey L we made all FEF injections before we began the DMFC injection experiment. We were concerned that possible damage incurred during the FEF injections may have somehow influenced the results of the DMFC injections. Therefore, in monkey I, we began the FEF and DMFC injections together. We generally alternated between injecting the DMFC of monkey I one day, then injecting into its FEF a few days later. In this way, we expect that if the eye fields incurred permanent damage from the injections, they would be affected relatively simultaneously. The results using monkey I were identical to that of monkey L, however, so we doubt that the influence of FEF damage on the DMFC's functions was a significant factor in our experiments.

Lidocaine injection method

See the accompanying FEF paper for details regarding the injection of lidocaine. The only difference between the FEF and DMFC injections was that of injection depth. Injections into the DMFC were shallower, aimed only 1 to 3 mm below the first unit, compared with the deeper FEF injections. This was because the DMFC is mostly in a gyrus and is presented normal to the track of a penetration, whereas the FEF is deeper, in the bank of a sulcus, and is presented obliquely or tangential to a penetration's track. The DMFC injection depth range coincides

with the range common to all DMFC sites within which saccades and fixations could be electrically elicited (from the first unit encountered to 4 mm deeper, see above). A range was used and not a single, precise depth, because the investigator had to explore a bit and find a stable, high-firing multiunit site for the accurate monitoring of inactivation. Occasionally, we injected shallower than 1 mm from the first unit, to try compensating for the inevitable depression of cortex during dural penetration and its subsequent slight "rebound" rise after the first unit is encountered. The outcome of such extra-shallow injections are described in RESULTS.

Visual stimulation and data collection

In summary, the monkeys faced a board of LEDs in complete darkness. During a task, one or more of the LEDs would be lit in sequence, controlled by a PDP-11 computer. Eye position data and task event information were stored by the computer at 333 Hz. The average multiple unit activity during every trial was calculated and stored by the computer. Task events during a trial were synchronized to the beginnings and endings of fixations at particular LEDs, as determined by on-line calculation of eye position and velocity.

Oculomotor tasks

The three basic tasks described in the accompanying FEF report were also used in this DMFC study. They are briefly reviewed below. An additional task was introduced, the "double-saccade" task, described next.

Memory target task: The monkey initially fixated an LED, which then remained lit while a peripheral LED was flashed. After the fixation LED disappeared, the monkey was required to make a saccade to the remembered location of the flashed LED.

Visual target task: The monkey initially fixated an LED, which then disappeared, and a second LED was lit. The monkey's task was simply to make a saccade as soon as possible to this second, "target", LED.

Fixation task: A single LED was lit somewhere in the visual field. The monkey had 5 seconds to foveate the LED, and then it had to fixate it steadily for 5 seconds in order to receive the full reward (breaking the fixation early resulted in delivery of only half the amount of juice).

Double-saccade task: This task was used to test the monkey's ability to make a sequence of saccades and fixations, rather than just a single saccade and fixation as tested in the first three tasks (Figure 2 A). A central LED was lit to start a trial. Soon after the monkey fixated this LED, it disappeared. Two targets were then flashed, the first for 110 msec and the second for 20 msec. These times were unequal because the monkey had a natural

tendency to go to the second, most recently flashed, target first even after extensive training; biasing the two targets temporally in favor of the first one allowed the monkey to perform the task at high proficiency (such that > 80% of trials were correctly performed). The two target flashes nearly always occurred before the monkey could make a saccade (reaction times were usually > 130 msec). The monkey was required to make saccades to the first and second target locations in the order that they were presented. The monkey had 400 msec to move after the targets were flashed; once it initiated a saccade it had to fixate within the window surrounding the first target within 200 msec; then it had to make a saccade into the second target's window within 800 msec. This task was done in dim room light to aid the monkey in this more complicated behavior and to avoid the upward drift of the eye normally seen in total darkness. The light did not illuminate the LED board directly. The LEDs could only be seen when lit.

Analysis

The methods of analyzing the visual target, memory target, and fixation tasks, as well as the analysis of eye movements during the inter-trial periods, were described in the FEF report. For this DMFC study, we analyzed the results of these single-saccade tasks by spatial quadrant, rather than by ipsi- or contralaterality, since the DMFC has been implicated in spatial

rather than retinotopic coding (see INTRODUCTION). The double-saccade task was analyzed as follows. Trials in which the monkey made a saccade before the first target was presented were aborted and not analyzed. For non-aborted trials, the first two eye movements following fixation LED offset were plotted as trajectories in 2-D space. Responses were classified into three types: sometimes a correct sequence was made (Figure 2 B), sometimes a sequence error was made (Figure 2 C), and sometimes no saccade was made within 400 msec (Figure 2 D).

Results

Overall injection results

We made 16 injections into the DMFC, all using lidocaine. Fifteen of these were using the single-saccade paradigms (9 in monkey L, 6 in monkey I), and 1 used the double-saccade paradigm (monkey I). Since there were negligible positive results during lidocaine injections using the single-saccade tasks, we did not do muscimol or saline injections using these tasks. The double-saccade task was affected by lidocaine injection, however, and so we plan to follow up the results reported here with more DMFC lidocaine injections, as well as muscimol and saline injections, in the upcoming months.

Figure 1 shows the estimated sites of penetration in the DMFCs of each monkey. These estimates are from surgical

observation of the midline's position as a mediolateral reference and from using the arcuate sulcus position, known from the FEF chamber implantation surgeries, as a rostrocaudal reference. Also, the estimates conform to the known position of the topographic map of termination zones (Tehovnik & Lee, 1993; Lee & Tehovnik, 1995). Only one injection was performed at each site. Histology is not yet available for these animals.

Single-saccade tasks

Memory target task

Inactivation of the DMFC did not cause any large or systematic deficits in the ability to generate saccades to remembered target locations. This robust negative result was found in 5 out of 5 lidocaine injections. As illustrated in Figure 3, the monkey performed as well during the DMFC multiunit shutdown as when the multiunits were firing vigorously (note that contralateral is now to the left, ipsilateral to the right, for all results of this paper).

Neither the saccadic error (Figure 4) nor the saccadic latency (Figure 5) to remembered targets were systematically affected by lidocaine inactivation, even when analyzed by quadrant. One-tailed, paired t-tests were used to compare the pre-inactivation ("before") data with results during and after the inactivation, as was done in the accompanying FEF study.

Some significant, small effects were seen, but these were particular to specific injections and were not repeated in others. Only one of these significant effects occurred during the inactivation, and it was actually a significant improvement of saccadic error over the baseline level (Figure 4 D, Q4). The other three significant effects occurred long after the inactivation (Figures 4 and 5). The frequency of premature responses was always low (< 20% of all responses) and was not affected by DMFC inactivation (not shown).

We were concerned that perhaps the negative results observed during DMFC inactivation were due to non-optimal placement of the needle tip in the cortex. It is well-known to neurophysiologists that introduction of an electrode through the dura into the brain causes a slight initial depression of cortex. Therefore, after one encounters the first unit, the cortex might rise up around the electrode tip. The tip would then be some depth below the surface, not at the surface, even though the electrode itself remained stationary. This phenomenon might cause our injections to be occurring deeper than we expected, and perhaps not enough grey matter was being inactivated to cause an effect.

To control for this possible complication, we varied the depth of needle tip placement for the five injections just considered in Figures 4 and 5. The electrode tip always moved with the needle tip so that the two were at equal depths. The results of this variation are shown in the inactivation curves of Figure 6. As the needle tip was placed shallower in the cortex

(from A to E in Figure 6), the inactivation occurred in a much briefer manner. This was probably due to more of the lidocaine escaping up the penetration shaft. Regardless of needle tip placement, there was no effect of DMFC inactivation on the memory target task (cf. Figures 4 and 5). Therefore, we do not attribute the negative results reported here to non-optimal needle tip depth placement.

Visual target task

No deficits were seen using this task, either. This was confirmed in 5 out of 5 lidocaine injections. An example of the negative results we saw is shown in Figure 7. The injection data for three of the injections, for which target duration was constant at 30 msec, are quantified in Figures 8 and 9. The other two injections used the paradigm, introduced in the FEF study, of varying target duration five-fold from 10 to 1000 msec; no effect was seen and the results are not shown. There were no changes in the frequency of trials in which no saccade, or tiny (< 2 deg.) saccades, were made in response to target onset during DMFC inactivation; frequency of such trials was essentially invariant at about 5%.

For the three experiments quantified in Figures 8 and 9, the initial position of the eye was varied randomly by trial, such that the monkey was fixating either contralaterally (left), centrally, or ipsilaterally (right). Regardless of the eye's

initial orbital position, no effect was seen during DMFC inactivation (Figure 10). This initial variation of eye position was also done in each of the 5 memory target tasks, also with no consequence.

Fixation task

4 out of the 5 lidocaine injections for which the fixation task was used failed to yield a deficit. One injection did seem to cause a deficit, but it had a much different nature than the fixation deficits seen with FEF inactivation. Its onset began with a rise in fixation error (Figure 11 D) just after the end of the lidocaine injection. Acquisition time rose (Figure 11 C) and acquisition percentage dropped sporadically (Figure 11 B) long after the end of the injection. These deficits were highly localized to one LED location (lower ipsilateral). The overall Fixation Deficit Index for this injection worsened with time and did not recover when the neurons did (Figure 12 A). This curious deficit had no parallel with any of the FEF inactivation deficits seen using this task. None of the other four DMFC inactivations using this task showed any hint of a deficit (Figure 12 B,C,D,E), either during or after the inactivation.

Finally, in the 5 memory target and 3 visual target tasks in which three initial fixation positions were used, there was never any indication of the monkey having a problem acquiring either the ipsilateral, central, or contralateral LED during the

inactivation. This was in marked contrast to the qualitative observation noted for FEF inactivation, and it reinforces the quantitative observation that fixation deficits following DMFC inactivation were rare.

Inter-trial intervals

In the 15 lidocaine injections into the DMFC, there was no trend for the eye's resting position in the dark to change its horizontal position (Figure 13 A). The average position before the injection was -6.13 deg. (SD, 2.38 deg.) and just after the end of the injection it was -5.89 deg. (SD, 2.06 deg.). This represents an ipsilateral (rightward) shift of 0.24 deg. (SD, 2.30 deg.) which was not significant ($t(14)$, $p = .3472$). Like the lidocaine and saline injections into the FEF, there was a slight decrease in the eye's vertical position after lidocaine injection into the DMFC (Figure 13 B): the mean vertical position changed from 7.76 deg. (SD, 2.91 deg.) to 7.14 deg. (SD, 2.61 deg.), a change of -0.625 deg. (SD, 0.904 deg.) that was significant ($t(14)$, $p = .009$).

Saccadic dynamics

The main sequences for saccades generated to all four quadrants from ipsilateral, central, and contralateral fixation during each of the single-saccade tasks were plotted, and no

systematic effects on dynamics could be found. An example of saccadic dynamics is shown in Figure 14. These main sequences (Figure 14) were derived in exactly the same way as those in the FEF study: initially ipsilateral fixation was used, only saccades with a contraversive component were included, and all the targets were contralateral. (The scatter patterns look slightly different from the ones in the FEF study because sometimes a more finely-space array of LEDs was used during FEF inactivation, to better probe the deficits). The main sequences during the DMFC inactivation were not significantly different from those derived from the data before and after the inactivations; in fact the regressions were nearly identical. This is true even if we zoom into the short-amplitude range (Figure 14 B and D) as was done with the FEF inactivation data.

Double-saccade tasks

DMFC inactivation

Right DMFC inactivation caused a clear, moderate, non-lateralized deficit in the monkey's ability to perform the double-saccade task. For illustration, we plot the first saccade made after the flashes of T1 and T2 (see Figure 2 for a review of this task). For example, look at Figure 15, panel Q4. Before the inactivation, most of the first saccades correctly entered the window surrounding T1. But during the inactivation, many of

the first saccades went elsewhere (specifically, many went to the second target, which was flashed (T2) in one of the other quadrants). The increase in frequency of sequence errors can be seen easily by looking at Figure 15 Q2 and Q1, too, but it is not as clear in Figure 15 Q3.

The effects of right DMFC inactivation on the double-saccade task are quantified in Figure 16. The percent correct dropped from 82% to 53% just after the injection (Figure 16 A), due to a parallel rise in the frequency of trials in which sequence errors or no saccades at all were made. The percent correct then steadily rose, to almost reach its baseline level by the end of the experiment.

The deficit affected each quadrant nearly equally (Figure 16 B, C, and D), although it was slightly milder for the lower contralateral quadrant (Q3). Percent correct dropped for each quadrant (Figure 16 B), the percent of sequence errors rose for all but quadrant Q3 (Figure 16 C), and the percent of times that no saccade was made rose for all quadrants (Figure 16 D) just after the injection.

FEF inactivation

Inactivation of the left FEF caused a severe deficit in the ability of the monkey to make saccades into the contralateral (righthand) quadrants using the double-saccade task. For example, look at Figure 17 Q4. Before the injection, the monkey

could easily saccade to T1 if it were presented in this lower contralateral (right) quadrant. During the inactivation, however, it made many fewer saccades into the window surrounding T1, and instead it often made sequence errors, making saccades directly to T2 (especially if T2 were ipsilateral, leftward). This effect was even greater for the upper contralateral (right) location of T1 (Figure 17 Q1). First saccades of the sequence made to T1 presented in ipsilateral (leftward) space were for the most part unaffected by left FEF inactivation (Figure 17 Q2 and Q3).

The lateralization of the deficit seen in the double-saccade task during left FEF inactivation is also obvious in the quantitative analyses (Figure 18). The percent correct declines most drastically for contralateral T1 locations (Figure 18 B), due to an especially sharp increase in sequence errors during such trials (Figure 18 C). Trials in which T1 was in the upper contralateral quadrant were particularly strongly affected in that, for over 40% of them, no saccade at all was made in response to the target flashes (Figure 18 D). There was a mild deficit for T1 presented in the ipsilateral quadrants Q2 and Q3; even though the monkey could make saccades to T1, it often could not complete the sequence by making a second saccade to T2 if T2 was presented contralaterally, in Q1 or Q4.

Discussion

Reversible inactivation of the DMFC failed to cause systematic deficits in the ability to make single saccades or fixations, but it did cause impairment in a saccadic sequence task. First we will address the negative results arising from the single-saccade tasks. Then we will discuss the significance of the double-saccade task deficits.

Memory target, visual target, and fixation tasks

Negative results of DMFC inactivation: We found no serious deficits in the ability to generate single saccades, tested with the memory target and visual target tasks, or fixations during DMFC inactivation. This suggests that, in the intact animal, the DMFC is not necessary for the generation of single saccades or fixations. The only severe deficit seen in this entire battery of tests was during one fixation task experiment, but the deficits in this experiment (Figure 11 and Figure 12 A) had a time-course that did not parallel the normal time-course of neural inactivation. Four other fixation task experiments showed no effect of DMFC inactivation.

We also found no effect of DMFC inactivation on 1) the occurrence of premature responses in the memory target task, 2) the occurrence of trials in which the eye failed to move during the visual target task, 3) the mean horizontal position of the

eye in darkness, and 4) the dynamics of saccades. In contrast, the accompanying report showed that FEF inactivation affects all of these. No effects of DMFC inactivation were revealed when the eye was initially positioned at different orbital positions, either.

Did we test the DMFC thoroughly enough? We tested each task in different areas of the DMFC of each monkey (Figure 1) to make sure each region of a large cortical area was adequately tested. We performed five injections with each task, which matched or exceeded the number of injections for each task in the accompanying FEF study. Also, we even tried varying our injection depth to allow for the possibility that we were not injecting at the optimal cortical position (Figure 6).

Were we injecting into the DMFC? Electrical stimulation throughout the injection region evoked saccades that landed in termination zones, and the termination zone sites followed the topography of Tehovnik & Lee (1993). One of the monkeys, L, had been used in a prior single-unit recording study (Lee & Tehovnik, 1995) and thus the fixation and saccade-related properties of this animal's DMFC had been well characterized. In both monkeys, we commonly noticed neural activity related to eye movements, fixations, or visual stimulation as recorded by the microelectrode, near the site of injection. Finally, from inspection of the locations of the midline and the arcuate sulci during surgeries, we believe that we were in the correct anatomical location (Tehovnik, 1995).

Were the data analyzed appropriately? Because it has been suggested that the DMFC uses a spatial, rather than a retinotopic vector code (Tehovnik & Lee, 1993; Tehovnik & Lee, 1995; Pierrot-Deseilligny et al., 1993), we even analyzed all the data in terms of spatial quadrant instead of visual hemifield. This analysis method did not reveal any patterns of impairment during DMFC inactivation. As could be observed by grouping the quadrants by hemifield in the figures, there was also no pattern of deficit with regard to contra- or ipsilaterality.

Might other single-saccade tasks reveal a deficit? It is possible that single-saccade tasks do exist that would suffer a deficit during DMFC inactivation. For example, it has been recently discovered that human lesions confined to the region corresponding to human DMFC or SMA are associated with an inability to make memory saccades using vestibular information only (Pierrot-Deseilligny et al., 1993). In that study a subject's head was rotated in the dark and then the task was to look back at the remembered starting point. Pierrot-Deseilligny et al. (1993) found that patients with FEF-area lesions could perform this single-saccade type of task, but those with DMFC-area lesions could not.

How do the present findings compare with previous results? The negative results that we found during DMFC inactivation are consistent with all previous DMFC lesion findings. Human DMFC lesions do not affect simple memory- and visually-guided saccade tasks (Pierrot-Deseilligny et al., 1991a,b; Gaymard et al.,

1990). The only tasks that have been found to necessitate an intact human DMFC are more complicated: the craniotopic memory saccade task (Pierrot-Deseilligny et al., 1993), as described above, and a task requiring sequences of two or three saccades (Gaymard et al., 1990). The limited DMFC lesion studies in monkey report similar findings with respect to forelimb reaching, another action that modulates DMFC neurons (Schall, 1991a; Mann et al., 1986): single reaches made by DMFC-lesioned monkeys in response to visual cues were normal, but more complex behaviors, such as self-paced movements, were impaired (Thaler, Chen, Nixon, Stern, & Passingham, 1995; Chen, Thaler, Nixon, Stern, & Passingham, 1995).

Double-saccade task

DMFC inactivation vs. FEF inactivation: DMFC inactivation impairs the ability to generate a sequence of two saccades. This was unexpected considering that DMFC inactivation has no effect on individual saccades or fixations. FEF inactivation also caused deficits in the double-saccade task, but this was no surprise, since the target flashes were very brief (T1 was 110 msec, T2 was 20 msec) and we have already shown that FEF inactivation severely impairs the execution of saccades to briefly flashed targets.

The double-saccade deficit during right DMFC inactivation was essentially omnidirectional. When the first target flash,

T1, was in three of the quadrants (the upper two and the lower right; Q1, Q2, and Q4, respectively), the drop in percent correct and the rise in each type of incorrect trial was about equal. The deficit was present, but was slightly less strong, for T1 in the lower left quadrant (Q3). Only further lidocaine injections, to be performed soon, will reveal whether this pattern is typical of DMFC inactivation.

In marked contrast, left FEF inactivation caused a highly lateralized effect: with T1 in the contralateral quadrants (upper and lower right, Q1 and Q4), the monkey's performance was abysmal. There was a slight impairment with T1 in the ipsilateral quadrants, too, during FEF inactivation. This was due to those trials in which T1 was ipsilateral but T2 was contralateral. Although ipsiversive saccades to T1 in the left quadrants could easily be made, the second, contraversive, saccades to T2 in the right quadrants were problematic because of the left FEF shutdown. A full analysis of the results with respect to second saccades and T2 locations is forthcoming but could not be completed in time for inclusion in this dissertation.

Comparison of these results with previous findings: These results confirm the human lesion study of Gaymard et al. (1990) that demonstrated a deficit in saccadic sequencing in patients with lesions of the DMFC region. That study found an omnidirectional deficit in the ability to make sequences of saccades, much like the effect observed during DMFC inactivation

in the present study (Gaymard et al., 1990). A later study (Rivaud, Muri, Gaymard, Vermersch, & Pierrot-Deseilligny, 1994) that tested patients with focal FEF lesions on the double-saccade task found lateralized deficits much like the ones reported here for monkey FEF inactivation: only contraversive saccades were impaired, whereas ipsiversive components of the sequences were unaffected.

Possible role of DMFC in the oculomotor system

Single-saccade tasks are impaired during FEF inactivation (see accompanying report) and during superior colliculus inactivation (Hikosaka & Wurtz, 1985a, 1986). Therefore, the FEF and SC seem to mediate basic saccadic generation, directly providing the brainstem saccadic generator with an E_d (desired eye position or displacement) signal and a trigger signal. However, single-saccade tasks are not affected by DMFC inactivation. In addition, a previous lesion study showed that bilateral ablation of both FEFs and both SCs eliminates voluntary saccade production, even though the DMFC remains intact (Schiller et al., 1980). These results suggest that the DMFC, in contrast to the FEF and SC, is not a direct generator of saccades. Yet a task that requires the planning of two saccades, the double-saccade task, is impaired when the DMFC is shut down. Therefore one role of the DMFC might be to act as a coordinator of movements that are directly commanded by the FEF and SC.

The DMFC is interconnected with both the FEF (Huerta et al., 1987; Schall et al., 1993) and the SC (Huerta & Kaas, 1990). It likely influences both the FEF and the SC: a recent study found that following ablation of either the FEF or SC alone, saccades could still be evoked from DMFC (Tehovnik, Lee, & Schiller, 1994). The omnidirectional deficits seen in the double-saccade task of the present study, and the ability to evoke both ipsi- and contraversive saccades from a single DMFC (Schlag & Schlag-Rey, 1987; Tehovnik & Lee, 1993), suggest that each DMFC has a bilateral influence on behavior. This is obviously advantageous for the general coordination of movement sequences. The bilaterality of DMFC might be due to the dense transcallosal projections between the DMFCs of the two hemispheres (Rouiller, Babalian, Kazennikov, Moret, Yu, & Wiesendanger, 1994).

Conclusion

DMFC inactivation did not cause severe deficits in the performance of single-saccade tasks and fixation tasks. We interpret these findings as indicating that in the intact animal, DMFC is not needed for simple saccade and fixation generation. These findings allow the results of higher-level oculomotor tasks to be better interpreted. Preliminary results using a saccadic sequence task indicate that DMFC is necessary for oculomotor planning. DMFC may exert a controlling influence over the more automatic saccade generators, FEF and SC.

Legends for Chapter 3

FIGURE 1: Approximate locations of right hemisphere DMFC penetrations with respect to the midlines (M) of each monkey and the principal (Ps) and arcuate (As) sulci (positions of these sulci were determined for the left hemisphere in the FEF study). The tasks used during lidocaine injection into each of these sites is shown. We tried to distribute the use of the single-saccade tasks such that the entire rostrocaudal extent of the topographic map (Tehovnik & Lee, 1993) would be tested evenly with the three tasks. The double-saccade task was tested once in the rostral region of the map (more experiments using this task are planned). Examples of stimulation-evoked saccades are shown on the far right: the eye is initially fixated in one of the positions shown by the large squares, then stimulation is applied. In the rostral DMFC (top) the stimulation causes a saccade to be made to the lower left, unless the eye is already in the lower left, in which case fixation is maintained. In the caudal DMFC (bottom), stimulation causes centering saccades, either ipsi- or contraversive.

FIGURE 2: (A) The double-saccade task consisted of two targets flashed in sequence, to which the monkey had to make saccades in order. 300 msec after start of fixation, the fixation LED was turned off and the first target (T1) was flashed for 110 msec,

then the second target (T2) was immediately flashed for 20 msec. The eye's typical response was to move after T2 was extinguished (schematized 1-D Eye trace, below). Actual 2-D eye trajectories illustrate the monkey's three types of response: (B) in a correct trial, the monkey made a saccade to the locations of T1 and T2 in the correct order in the allotted time; (C) often "sequence errors" occurred in which the monkey did not go to T1 and then T2 in order; (D) sometimes no saccade was made in the allotted response time.

FIGURE 3: Trajectories of saccades made during the memory tasks were unaffected by DMFC inactivation. Saccadic trajectories superimposed from trials (A) before, (B) during, and (C) after inactivation. Symbols and conventions described in Figure 2 of the FEF paper.

FIGURE 4: Saccadic error before, during, and after DMFC inactivation for the memory target task (cf. Figure 6 of FEF paper, left column; ordinate scaling is the same). (A), (B), (C), (D), and (E) represent five different lidocaine injections into the DMFC. The contralateral quadrants, Q2 and Q3, are on the left and the ipsilateral quadrants, Q1 and Q4, are on the right (see quadrants layout in lower right). Statistically, the values during and after inactivation are each compared with the values before inactivation. The only significant changes were small decreases in saccadic error in (D), ipsilaterally.

FIGURE 5: Saccadic latency before, during, and after DMFC inactivation (cf. Figure 6 of FEF paper, right column; scale is same). Same injections (A) - (E), as shown in Figure 4. In the two contralateral quadrants of (B) there were slight but significant increases in latency, but these were uncorrelated with neural shutdown.

FIGURE 6: The depth of injection for the experiments shown in the Figures 4 and 5 was actually varied to account for possible depression of cortex during needle and electrode insertion, and its possible subsequent rise. Multiunit inactivation curves are shown for five different depths of injection, from 2.44 mm below the first unit (A) to 0.05 mm below the first unit (E), as measured with a descending electrode (Depth (down)). (A) - (E) in this figure correspond to (A) - (E) in Figures 4 and 5; the shaded time periods in this figure are the times in which "during" data were collected for Figures 4 and 5. For the two extremely shallow injections, (D) and (E), the last unit encountered upon electrode withdrawal was also noted (Depth (up)). The difference between cortical surface estimates going down versus going up suggest that the cortical surface rose slightly around the electrode and needle. The rise was 1.0 mm in (D) and 0.80 mm in (E). Regardless of needle tip depth, there were no effects of DMFC inactivation using the memory task (See (A) - (E) of Figures 4 and 5).

FIGURE 7: Lack of effect of DMFC inactivation on trajectories made in the visual target task (30 msec target duration). Despite effective DMFC shutdown, the monkey could make saccades to targets presented anywhere in the visual field. The trajectories are less dense in (A) than in (B) and (C) because half as many trials were run in (A) as in (B) or (C). See Figure 3 for other figure explanations.

FIGURE 8: Saccadic error for three lidocaine injections, (A) - (C), using the visual target task (30 msec target duration). Only one significant, small increase in error was seen during the DMFC inactivation (A, Q2). The small magnitude of this deficit can be appreciated in the trajectory plot of Figure 7, from which the data of (A) were derived (compare the upper left quadrant of Figure 7 B, during the inactivation, with the upper left quadrant of Figure 7 A, before the inactivation).

FIGURE 9: Saccadic latency for the three lidocaine injections shown in the previous figure. The only significant, small effects are not correlated with DMFC inactivation, but occur long after it.

FIGURE 10: There was no orbital dependence of the monkey's ability to make saccades to 30 msec flashed targets during DMFC inactivation (cf. Figure 13 of the FEF paper). These are the same data as partially presented in Figure 7.

FIGURE 11: The one (out of 5) DMFC inactivation in which there was a deficit using the fixation task. The deficit was localized to the lower ipsilateral LED (white circle, thin solid line). Effects increased over time after the injection and did not seem correlated with the inactivation itself. Compare with the highly synchronous deficits seen in acquisition percentage, acquisition time, and fixation error that were typical of FEF inactivation (see Figure 15 B, C, and D of FEF paper). None of the other four DMFC inactivations showed deficits in any of these three measures. (See legend of Figure 15, FEF paper, for explanation of symbols).

FIGURE 12: Fixation Deficit Index (FDI) before, during, and after DMFC inactivation for all 5 fixation task experiments. Only one (A), which was also illustrated in the previous figure, showed a deficit. This deficit was actually worse after the inactivation than during the inactivation (cf. Figure 16 of the FEF paper). The other four experiments, (B) - (E), showed no deficit.

FIGURE 13: Eye position in the dark, during the inter-trial periods of all 15 lidocaine injections into the DMFC. (A) There is no significant, systematic shift in horizontal eye position after the end of injection compared with before the injection. (B) There is a very slight but significant decrease in the vertical eye position after the injection. For comparison with

FEF inactivation, see Figure 19 of the FEF paper.

FIGURE 14: Saccadic dynamics do not change during the DMFC inactivation as compared with dynamics before and after the inactivation. This is true for (A), (C), all amplitude ranges, including (B), (D), the short-amplitude range for which the deficit during FEF inactivation was found. See text for details.

FIGURE 15: Deficit during right DMFC inactivation while the monkey performs the double-saccade task. The first saccade of the sequence for each trial is plotted, and results from all trials in this experiment are superimposed. Data are separated by the quadrant (Q1, Q2, Q3, or Q4) in which T1 was flashed; window around T1 is shown. T2 for each trial was flashed in one of the other 3 quadrants, as depicted in panel Q4; saccades to T2 are not shown in this figure. The first saccades of the sequence Before DMFC inactivation are shown on the left in each panel, and those made During inactivation are on the right of each panel. The first saccade goes to the wrong location more often During the injection than Before, especially for T1 flashed in Q1, Q2, or Q4. Every first saccade that does not go to T1 indicates that a sequence error occurred. Other types of errors were made but are not shown in this figure, e.g. sometimes the monkey made a correct saccade to T1 but not to T2, or made no saccade at all.

FIGURE 16: Quantification of the deficit seen using the double-saccade task with right DMFC inactivation. (A) The overall percent correct drops just following the injection, due to a parallel elevation in the frequency of sequence errors and the frequency of trials in which no saccade was made. This effect recovers later. (B) The percent correct, separated by the quadrant in which T1 is flashed (see legend in lower right). The percent correct declines nearly equally after the injection regardless of where T1 is flashed. (C) Similarly, the frequency of sequence errors rises for T1 in any quadrant, as does the (D) frequency of trials in which no saccade is made.

FIGURE 17: Left FEF inactivation using the double-saccade task. Strong contralateral deficits were seen. First saccades of the sequence are normal Before the inactivation with T1 flashed in any quadrant, Q1 - Q4. During the inactivation, if T1 was flashed in the contralateral (right) quadrants (Q1 and Q4), the first saccades of the sequence were highly dysmetric. This resulted in elevated frequencies of sequence errors. In contrast, saccades are hardly affected During the inactivation if T1 is flashed in either of the ipsilateral (left) quadrants (Q2 and Q3). See legend of Figure 15 and text for details.

FIGURE 18: Extreme lateralization of the double-saccade task deficits during left FEF inactivation. (A) The overall percent correct plunges during the inactivation and then recovers, due

more to an increase in sequence errors than in the frequency at which no saccades are made. (B) Just after the FEF injection, the percent correct in trials for which T1 was contralateral (Q1 or Q4, dark symbols) is less than 20%, much worse than for trials in which T1 is ipsilateral (Q2 or Q3, white symbols). (C) Frequency of sequence errors is much worse for T1 in contralateral space, and (D) frequency at which no saccades are made after the targets are flashed is especially elevated for the upper contralateral T1 location (dark circle).

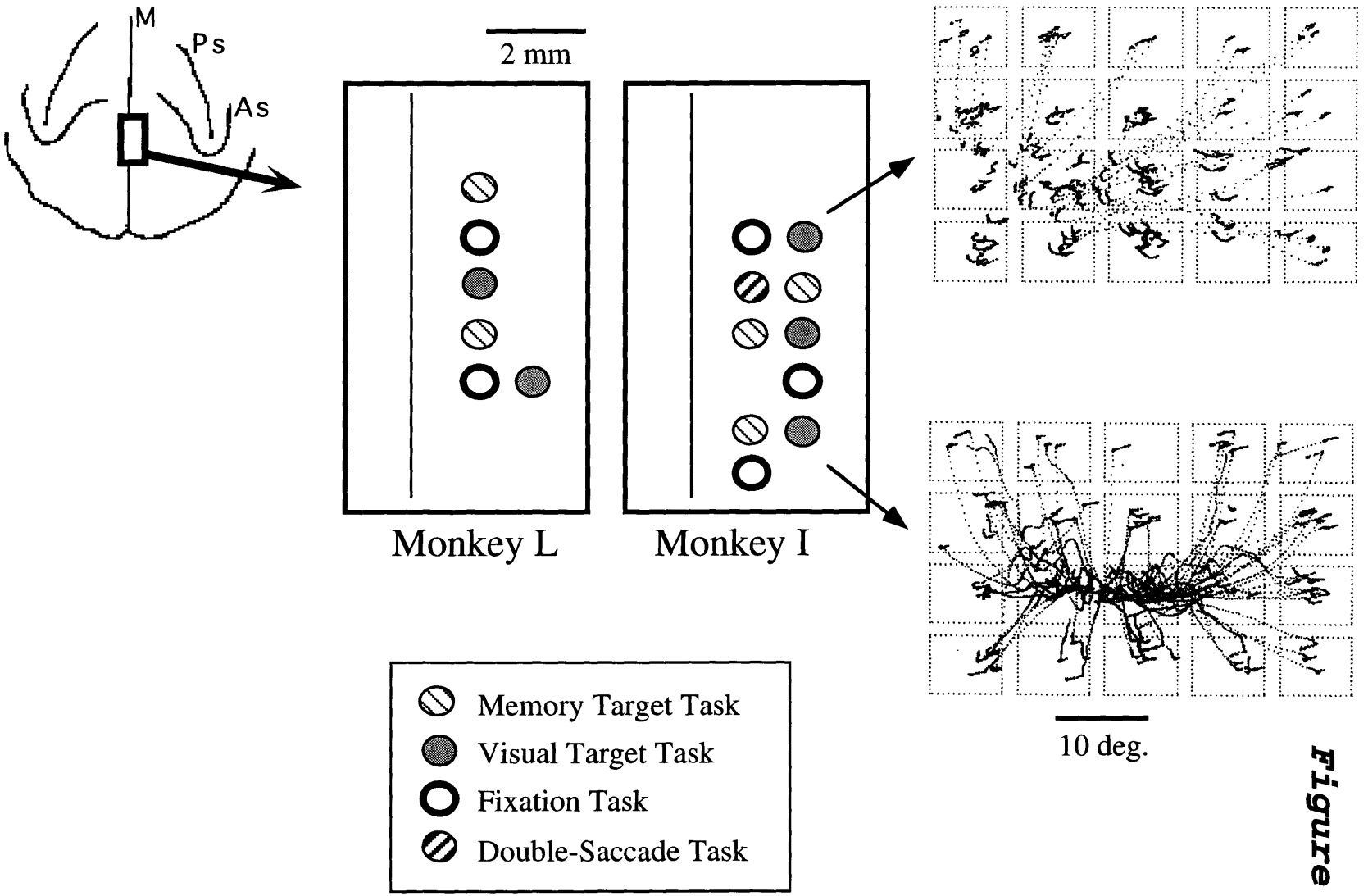
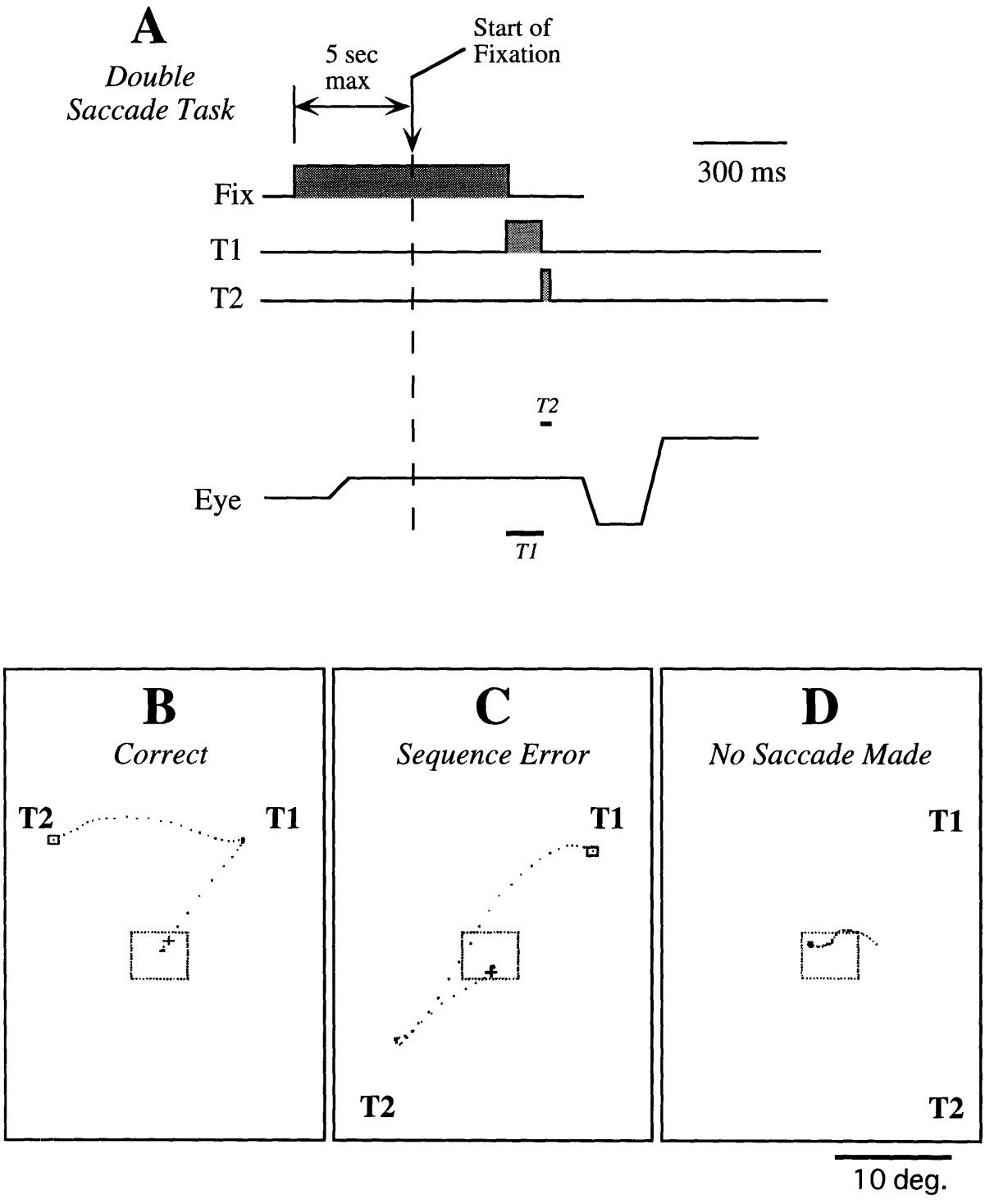

Anterior
M
Ps
As
2 mm
Monkey L
Monkey I
10 deg.
Memory Target Task
Visual Target Task
Fixation Task
Double-Saccade Task

Figure 1

Figure 2


A
Double Saccade Task
5 sec max
Start of Fixation
300 ms
Fix
T1
T2
T2
Eye
T1
B
Correct
T2
T1
C
Sequence Error
T1
T2
D
No Saccade Made
T1
T2
10 deg.

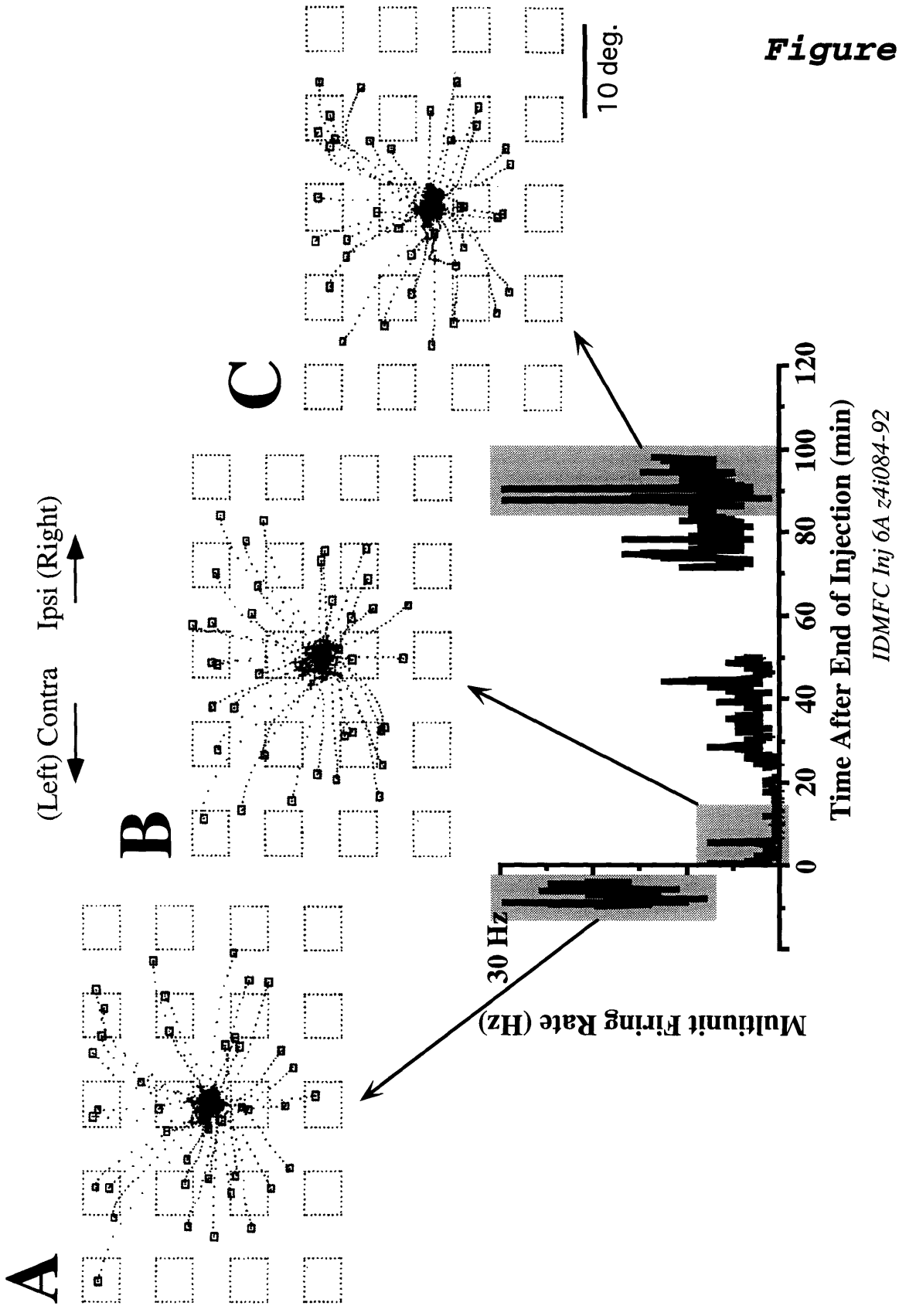

A
B
C
(Left) Contra
Ipsi (Right)
10 deg.
Multiunit Firing Rate (Hz)
30 Hz
0
20
40
60
80
100
120
Time After End of Injection (min)
IDMFC Inj 6A z4i084-92

Figure 3

Figure 4

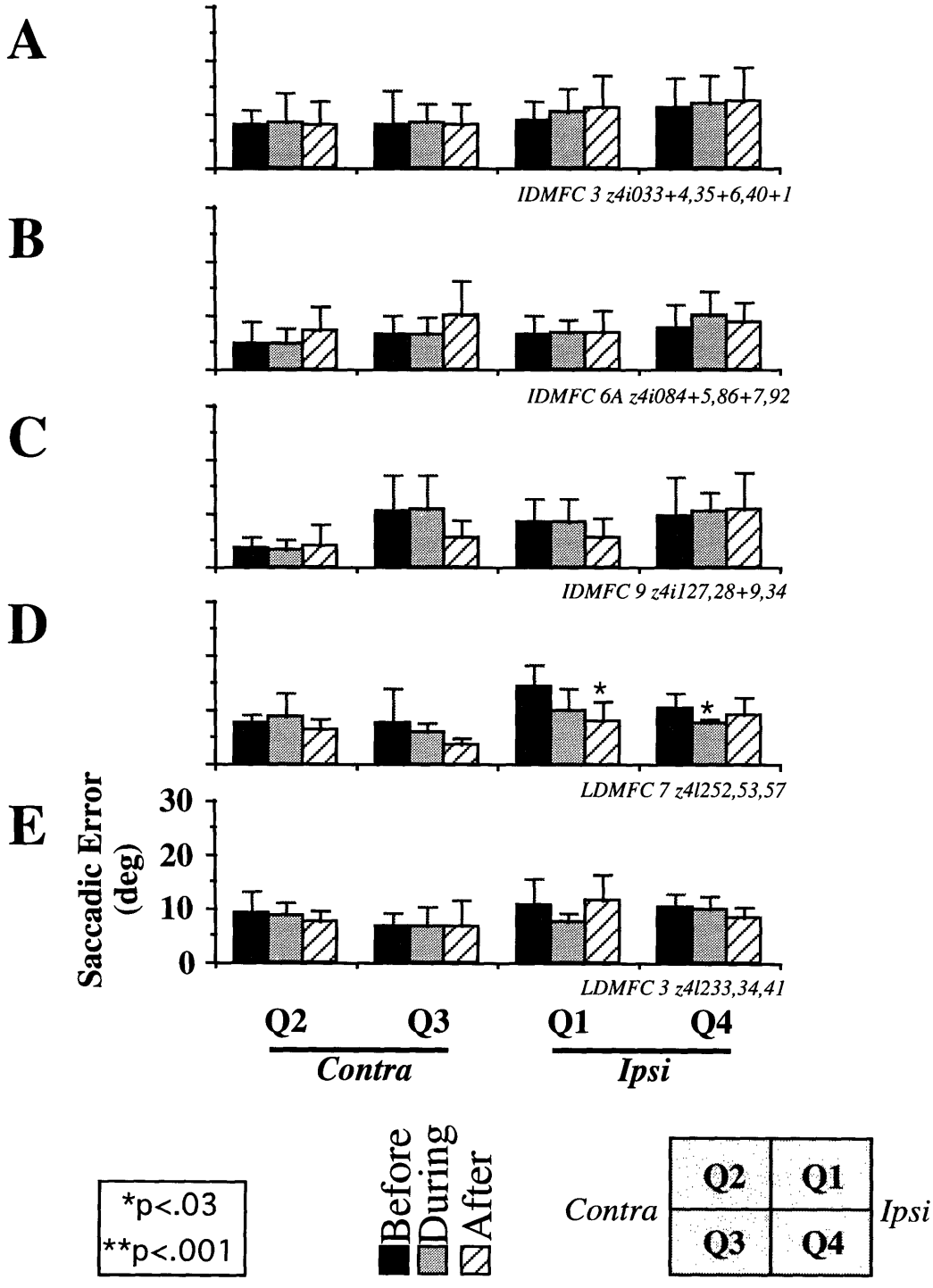

A
B
C
D
E
IDMFC 3 z4i033+4,35+6,40+1
IDMFC 6A z4i084+5,86+7,92
IDMFC 9 z4i127,28+9,34
LDMFC 7 z4l252,53,57
LDMFC 3 z4l233,34,41
Saccadic Error (deg)
30
20
10
0
Q2
Q3
Q1
Q4
Contra
Ipsi
*p<.03
**p<.001
Before
During
After
Contra
Q2
Q1
Q3
Q4
Ipsi

Figure 5

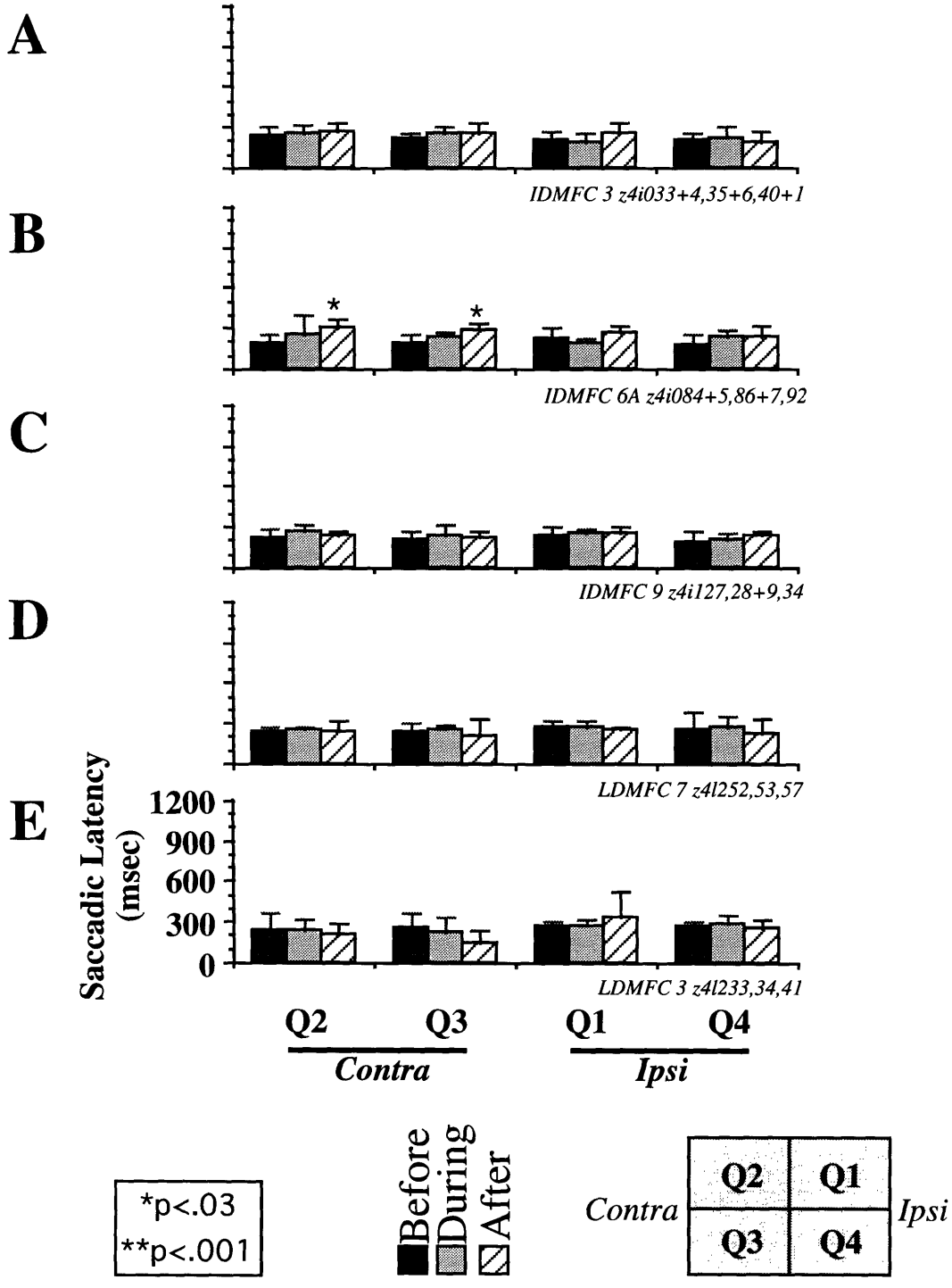
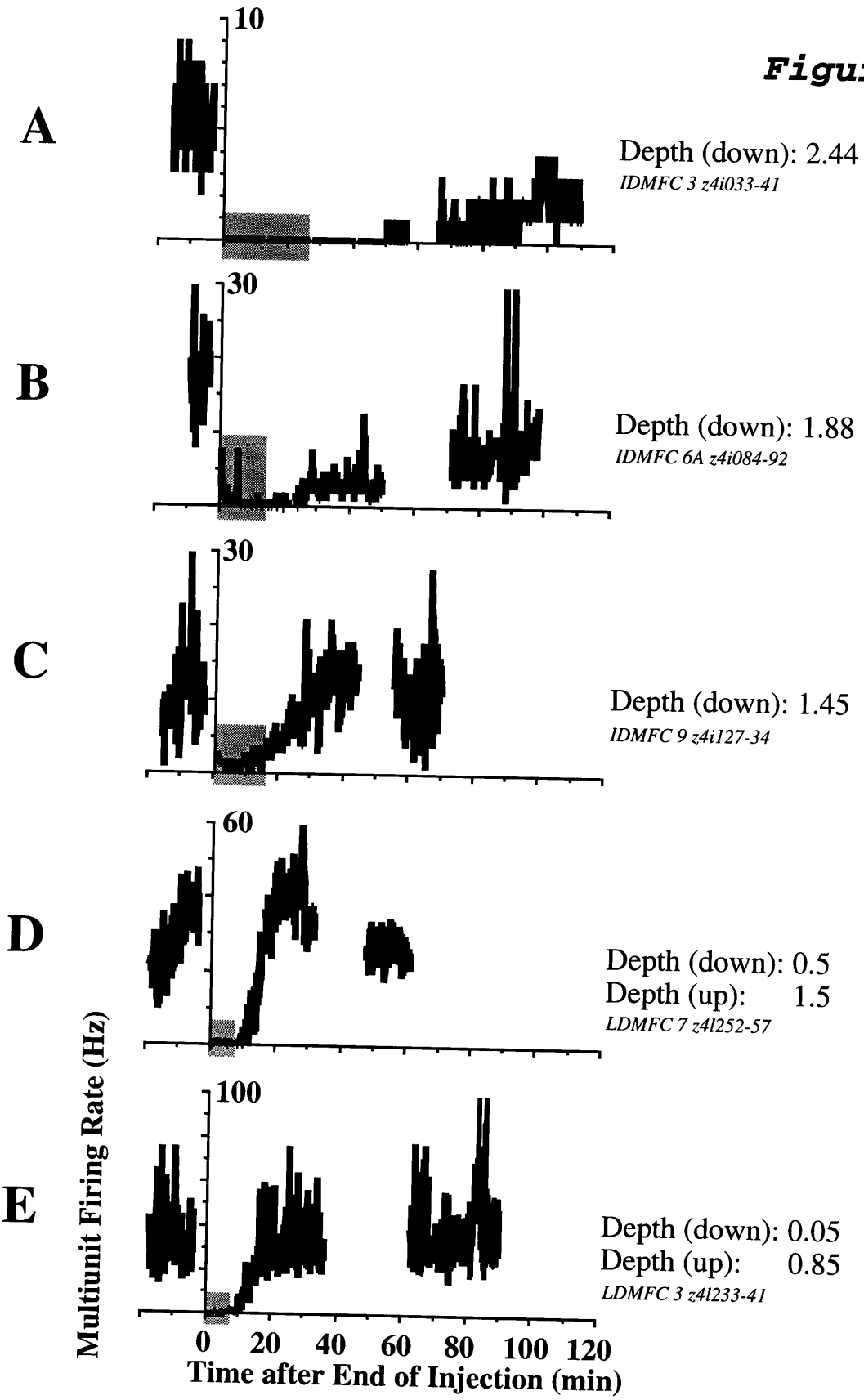

A
B
C
D
E
IDMFC 3 z4i033+4,35+6,40+1
IDMFC 6A z4i084+5,86+7,92
IDMFC 9 z4i127,28+9,34
LDMFC 7 z4l252,53,57
LDMFC 3 z4l233,34,41
Saccadic Latency (msec)
1200
900
600
300
0
Q2
Q3
Q1
Q4
Contra
Ipsi
*p<.03
**p<.001
Before
During
After
Contra
Q2
Q1
Q3
Q4
Ipsi

Figure 6


A
10
Depth (down): 2.44
IDMFC 3 z4i033-41
B
30
Depth (down): 1.88
IDMFC 6A z4i084-92
C
30
Depth (down): 1.45
IDMFC 9 z4i127-34
D
60
Depth (down): 0.5
Depth (up): 1.5
LDMFC 7 z4l252-57
E
100
Depth (down): 0.05
Depth (up): 0.85
LDMFC 3 z4l233-41
Multiunit Firing Rate (Hz)
0 20 40 60 80 100 120
Time after End of Injection (min)

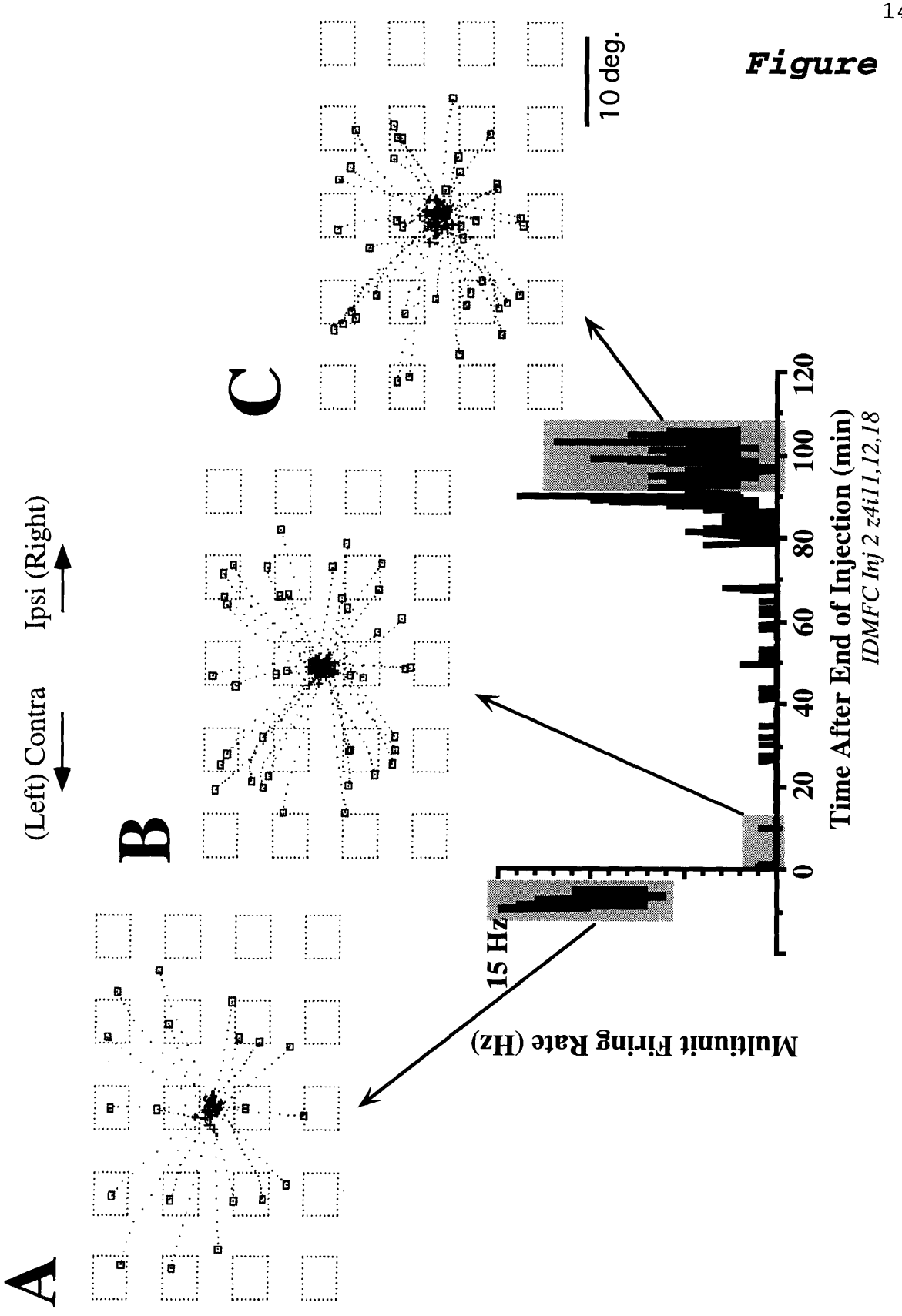

A
B
C
(Left) Contra
Ipsi (Right)
10 deg.
15 Hz
Multiunit Firing Rate (Hz)
0
20
40
60
80
100
120
Time After End of Injection (min)
IDMFC Inj 2 z4i11,12,18

Figure 7

Figure 8

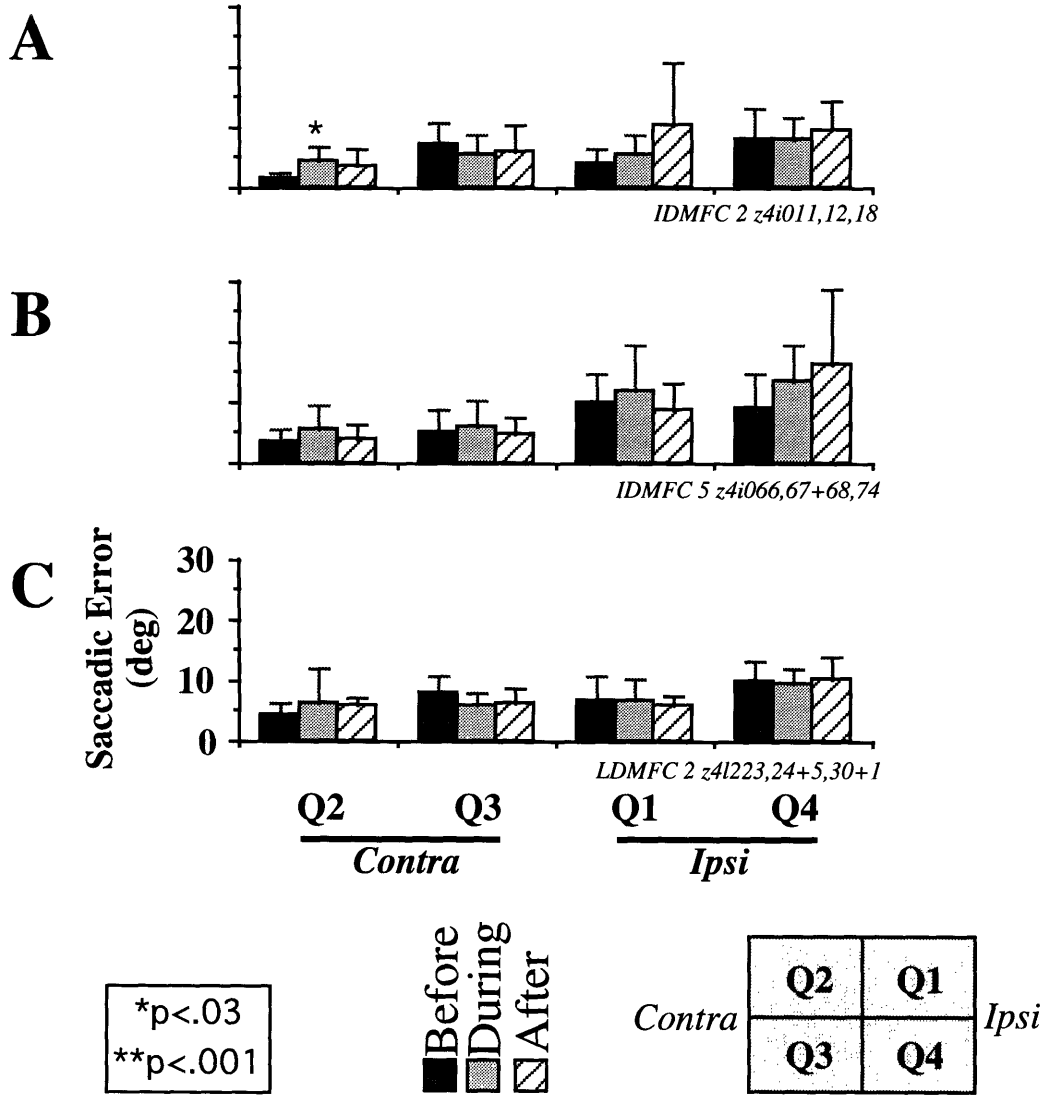

A
IDMFC 2 z4i011,12,18
B
IDMFC 5 z4i066,67+68,74
C
Saccadic Error (deg)
30
20
10
0
LDMFC 2 z4l223,24+5,30+1
Q2
Q3
Q1
Q4
Contra
Ipsi
*p<.03
**p<.001
Before
During
After
Contra
Q2
Q1
Q3
Q4
Ipsi

Figure 9

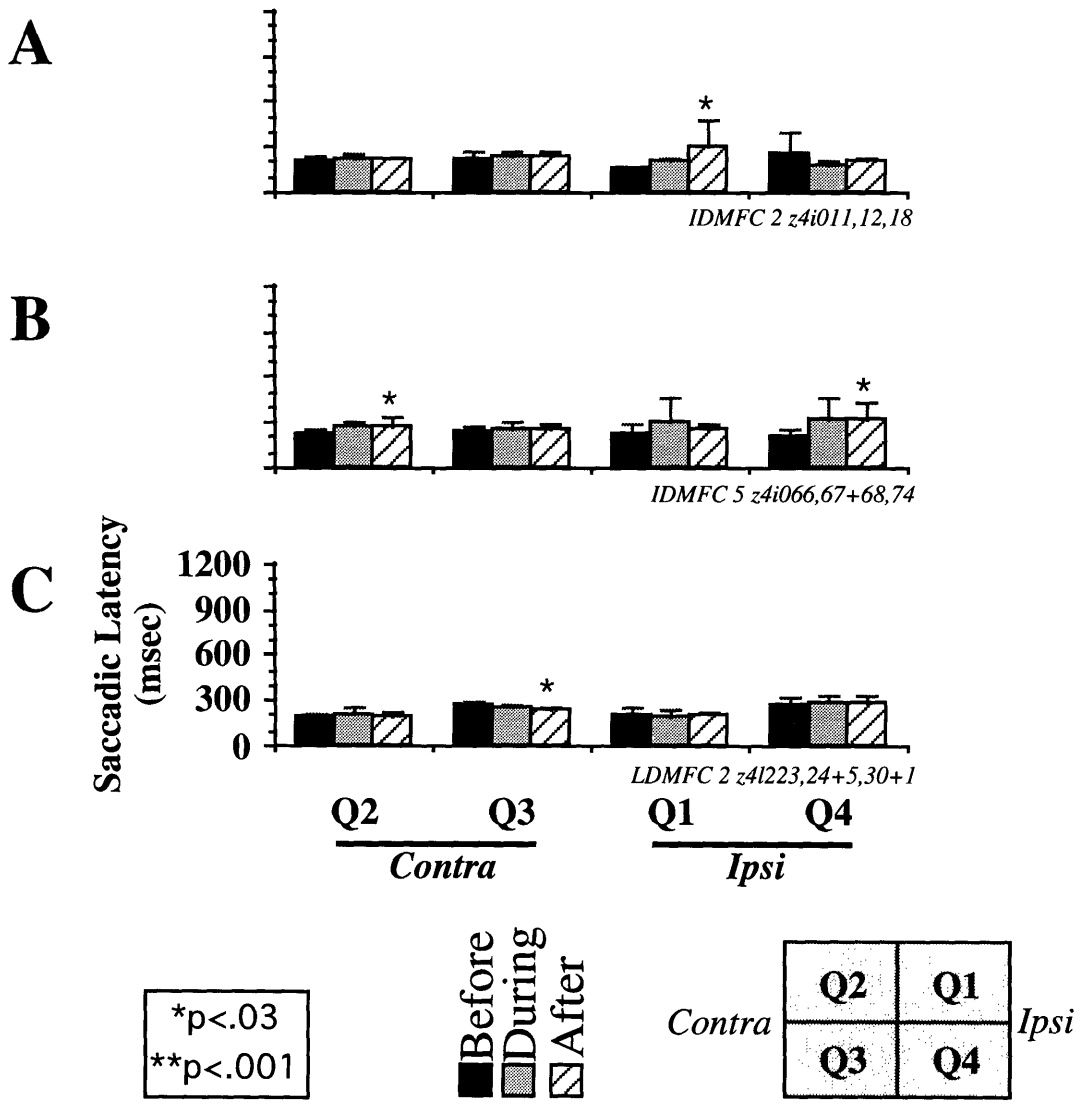

A
*
IDMFC 2 z4i011,12,18
B
*
*
IDMFC 5 z4i066,67+68,74
C
Saccadic Latency (msec)
1200
900
600
300
0
*
LDMFC 2 z4l223,24+5,30+1
Q2
Q3
Q1
Q4
Contra
Ipsi
*p<.03
**p<.001
Before
During
After
Contra
Q2
Q1
Q3
Q4
Ipsi

Figure 10

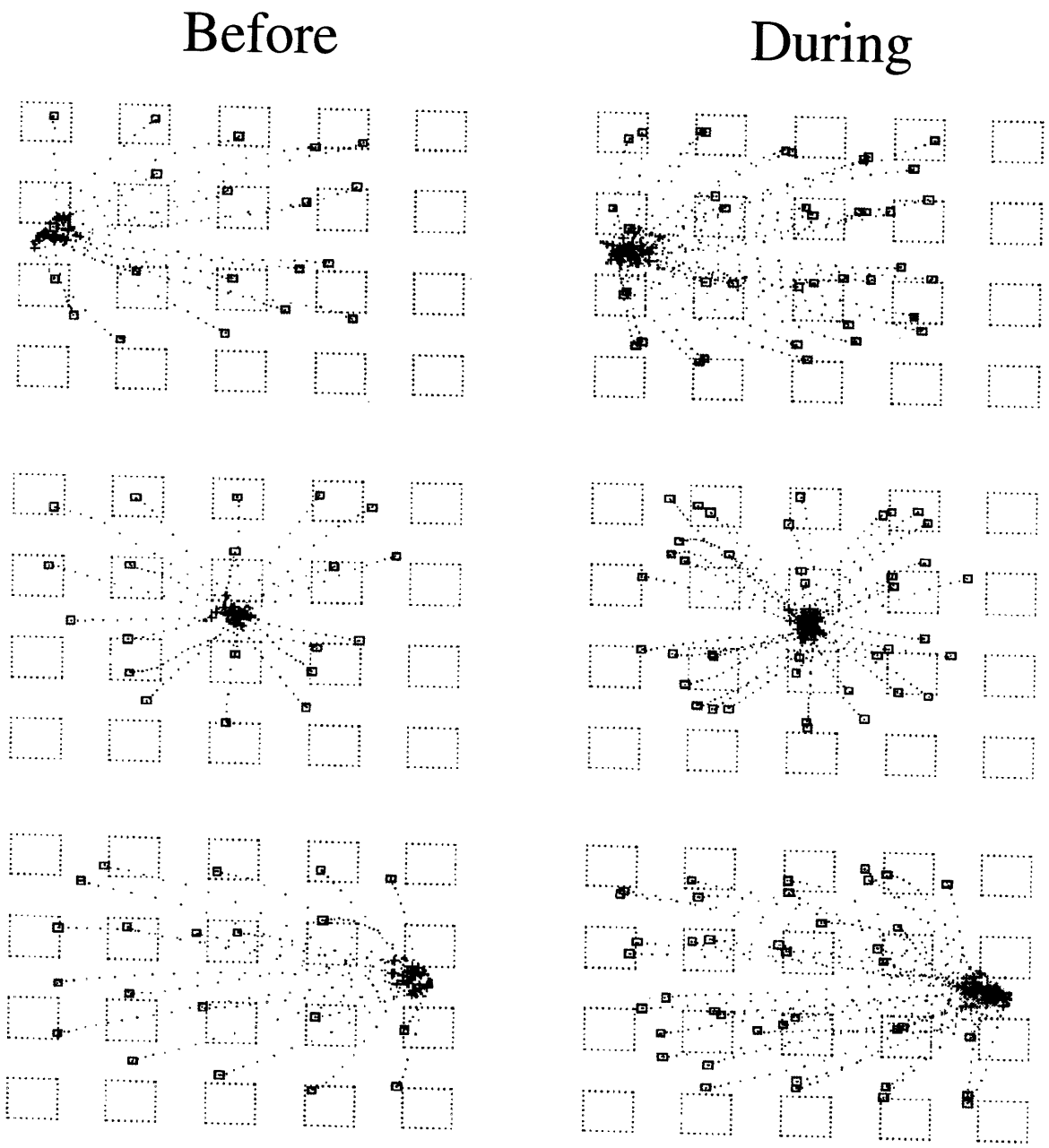


Figure 11

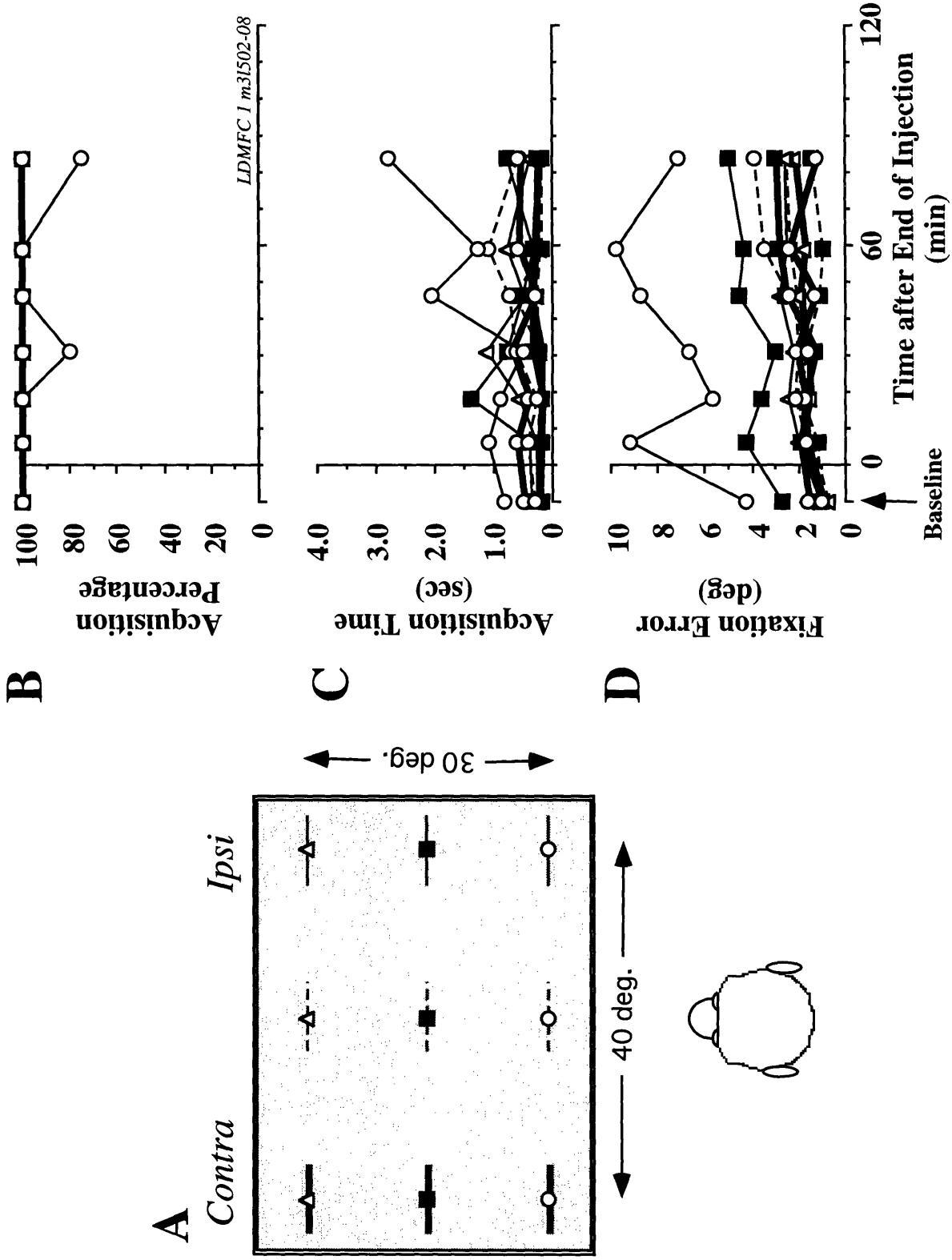

A
Contra
Ipsi
30 deg.
40 deg.
B
Acquisition Percentage
100
80
60
40
20
0
LDMFC 1 m3l502-08
C
Acquisition Time (sec)
4.0
3.0
2.0
1.0
0
D
Fixation Error (deg)
10
8
6
4
2
0
Baseline
0
60
120
Time after End of Injection (min)

Figure 12

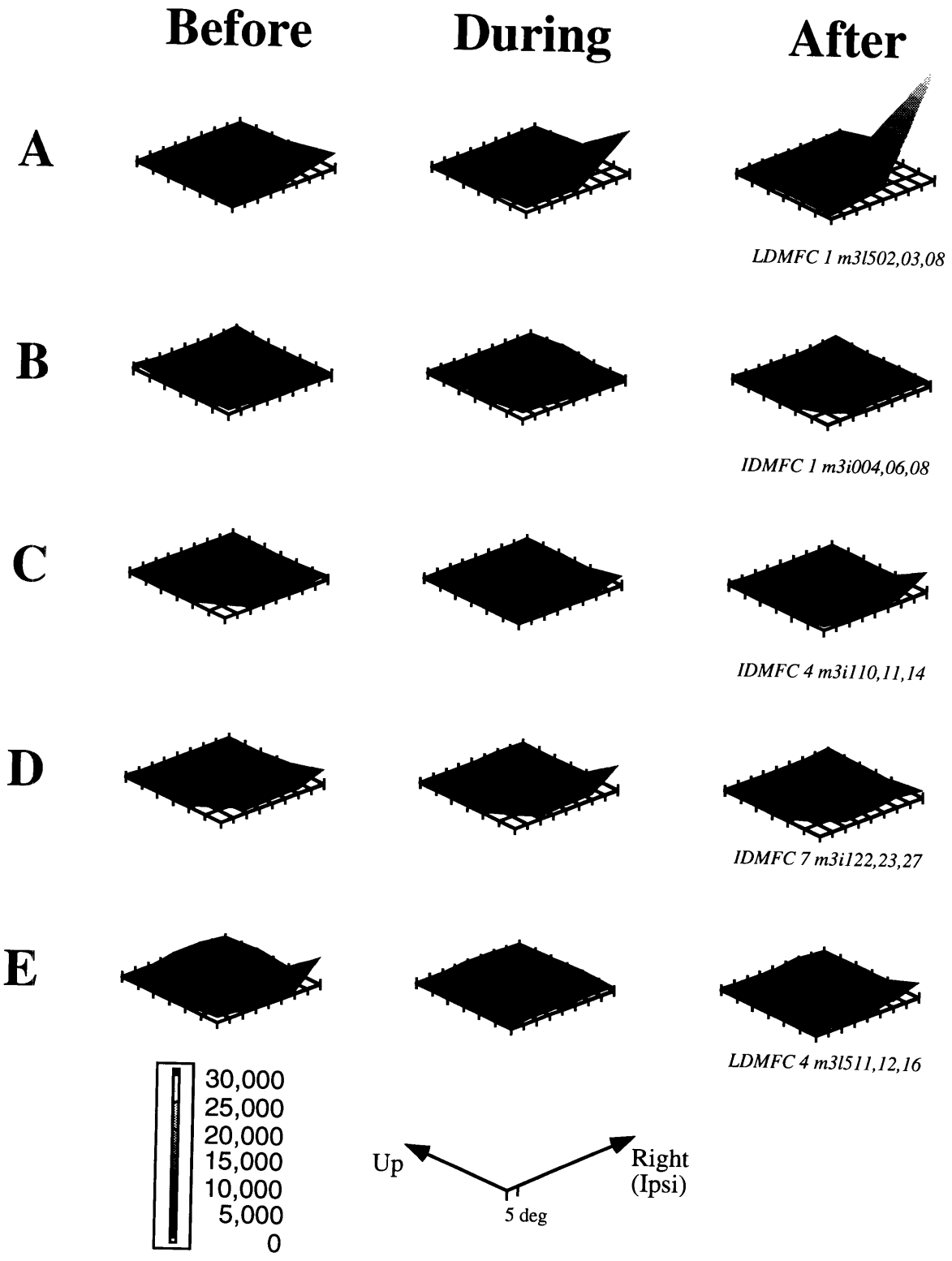

Before
During
After
A
B
C
D
E
LDMFC 1 m3l502,03,08
IDMFC 1 m3i004,06,08
IDMFC 4 m3i110,11,14
IDMFC 7 m3i122,23,27
LDMFC 4 m3l511,12,16
30,000
25,000
20,000
15,000
10,000
5,000
0
Up
Right
(Ipsi)
5 deg

Figure 13

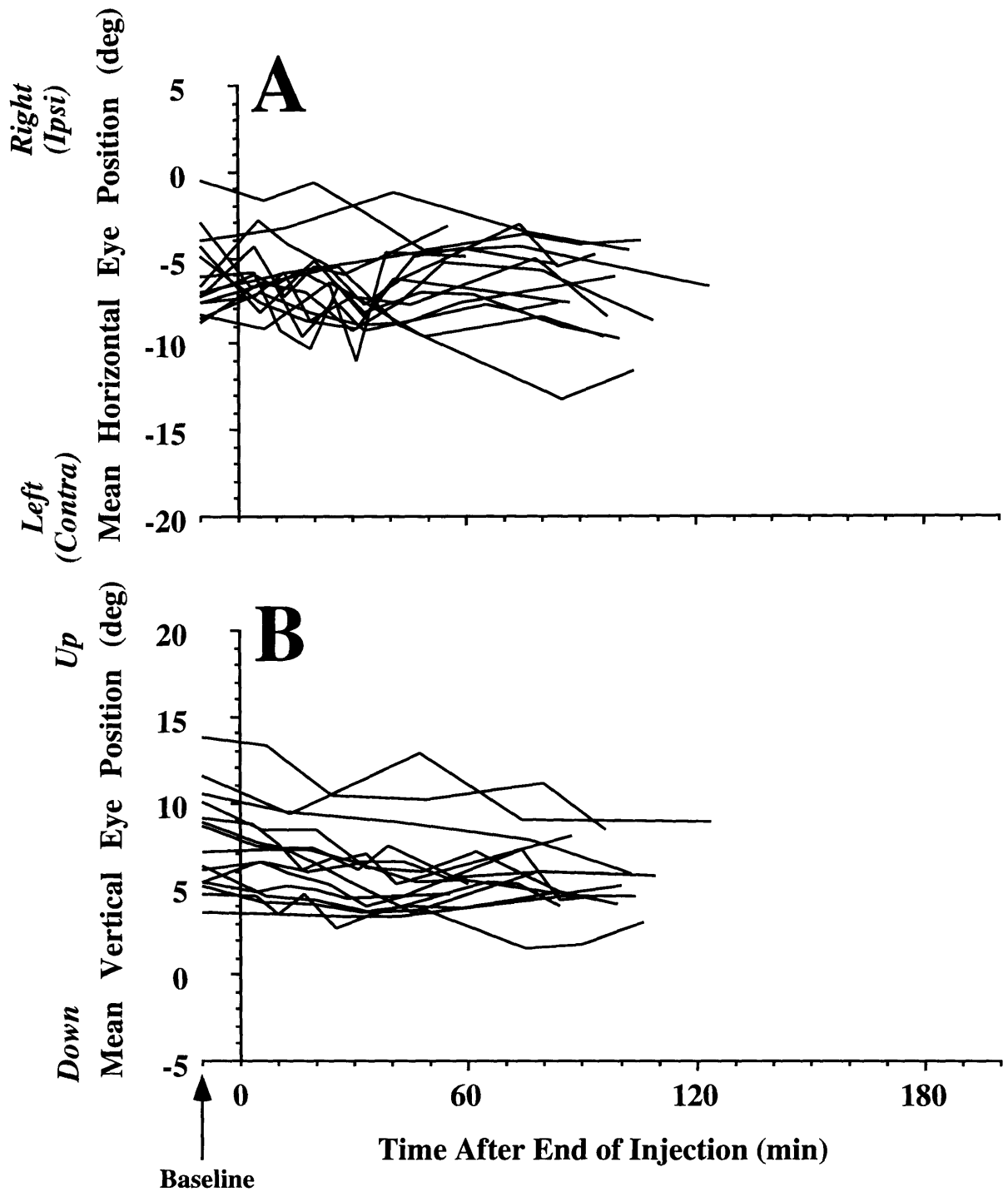
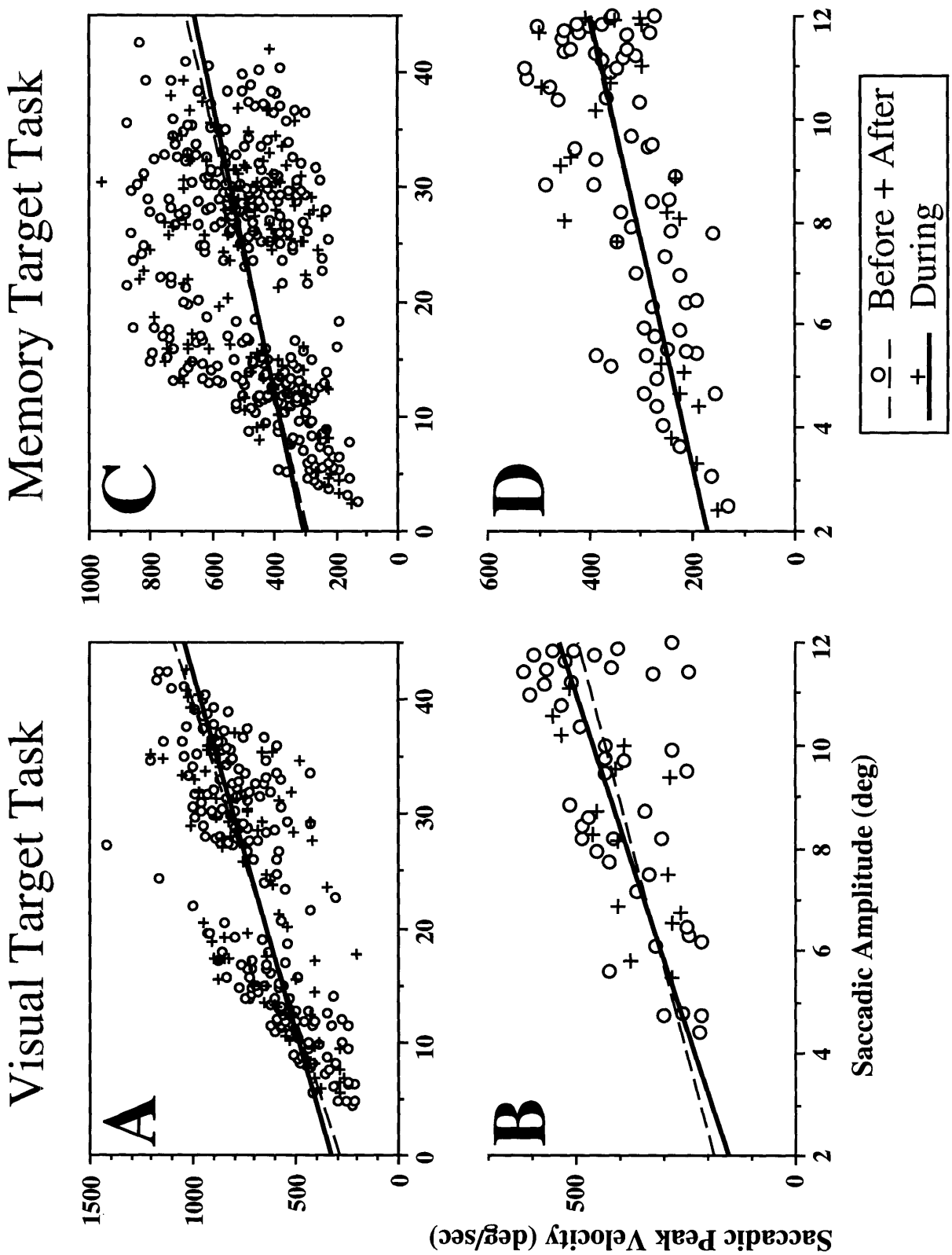
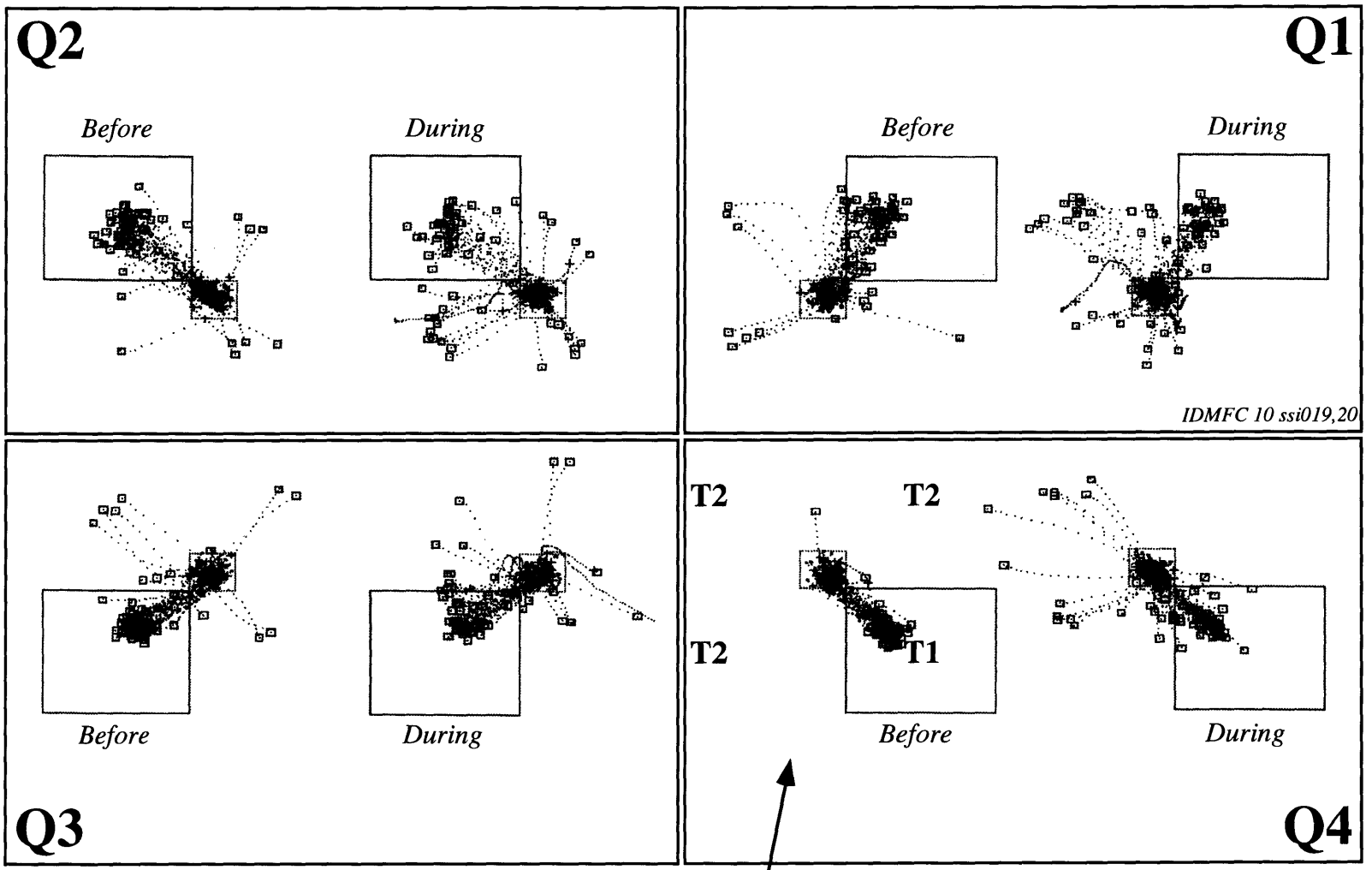

A
Right (Ipsi)
Left (Contra)
Mean Horizontal Eye Position (deg)
5
0
-5
-10
-15
-20
B
Up
Down
Mean Vertical Eye Position (deg)
20
15
10
5
0
-5
0
60
120
180
Time After End of Injection (min)
Baseline

Figure 14


Visual Target Task
Memory Target Task
A
B
C
D
Saccadic Peak Velocity (deg/sec)
Saccadic Amplitude (deg)
Before + After
During

First saccades of the sequence, *Before* and *During* right DMFC inactivation



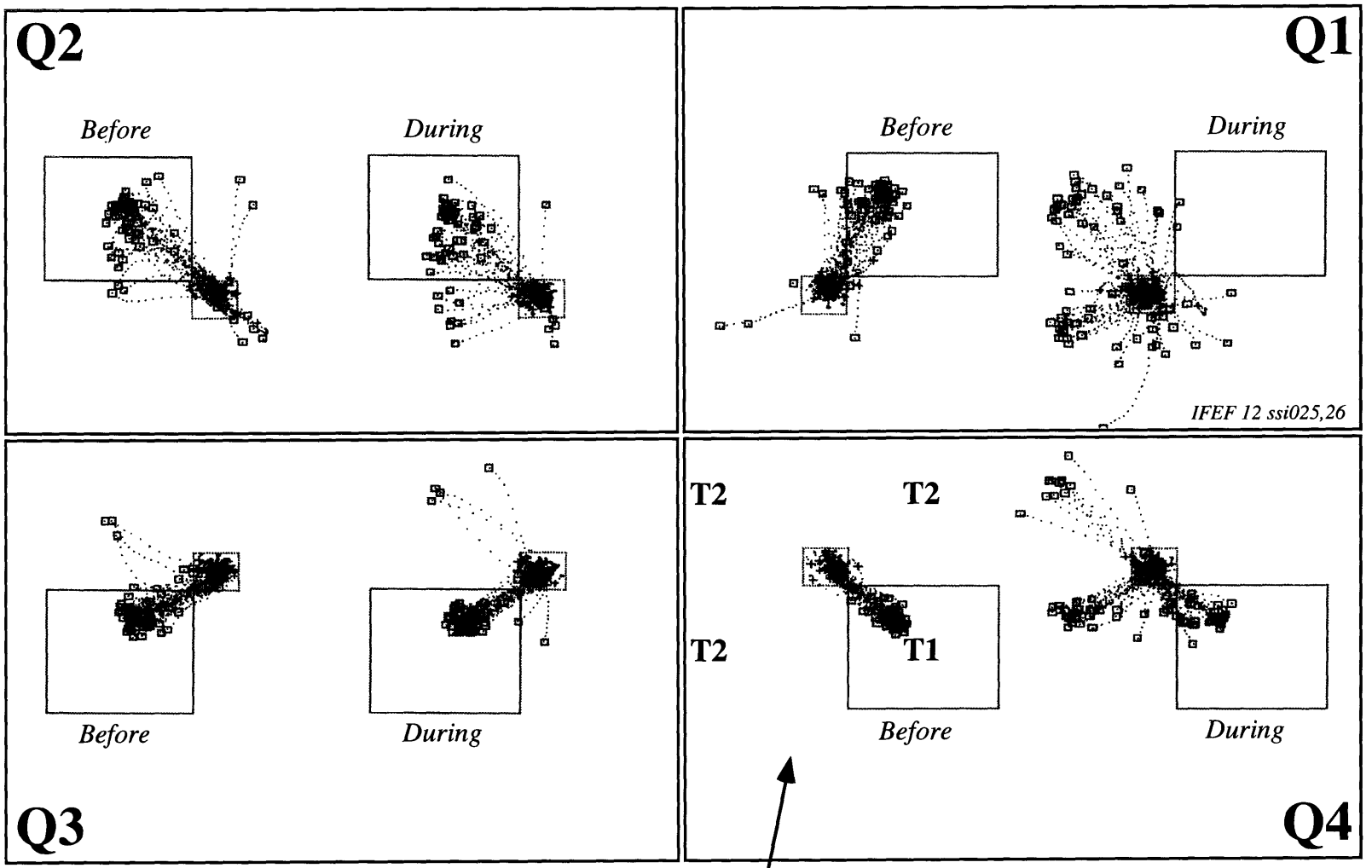
T1 is in center of window,
T2 is in one of the other quadrants

Figure 15

Figure 16

A
% Correct
% Sequence Errors
% No Saccade Made
Percent of Attempts
B
Percent Correct
100
80
60
40
20
0
0
20
40
60
80
100
Time after End of Injection (min)
C
Percent Sequence Errors
D
Q1 (Ipsi)
Q2 (Contra)
Q3 (Contra)
Q4 (Ipsi)
IDMFC 10 ssi019-23
Percent No Saccades Made
Baseline

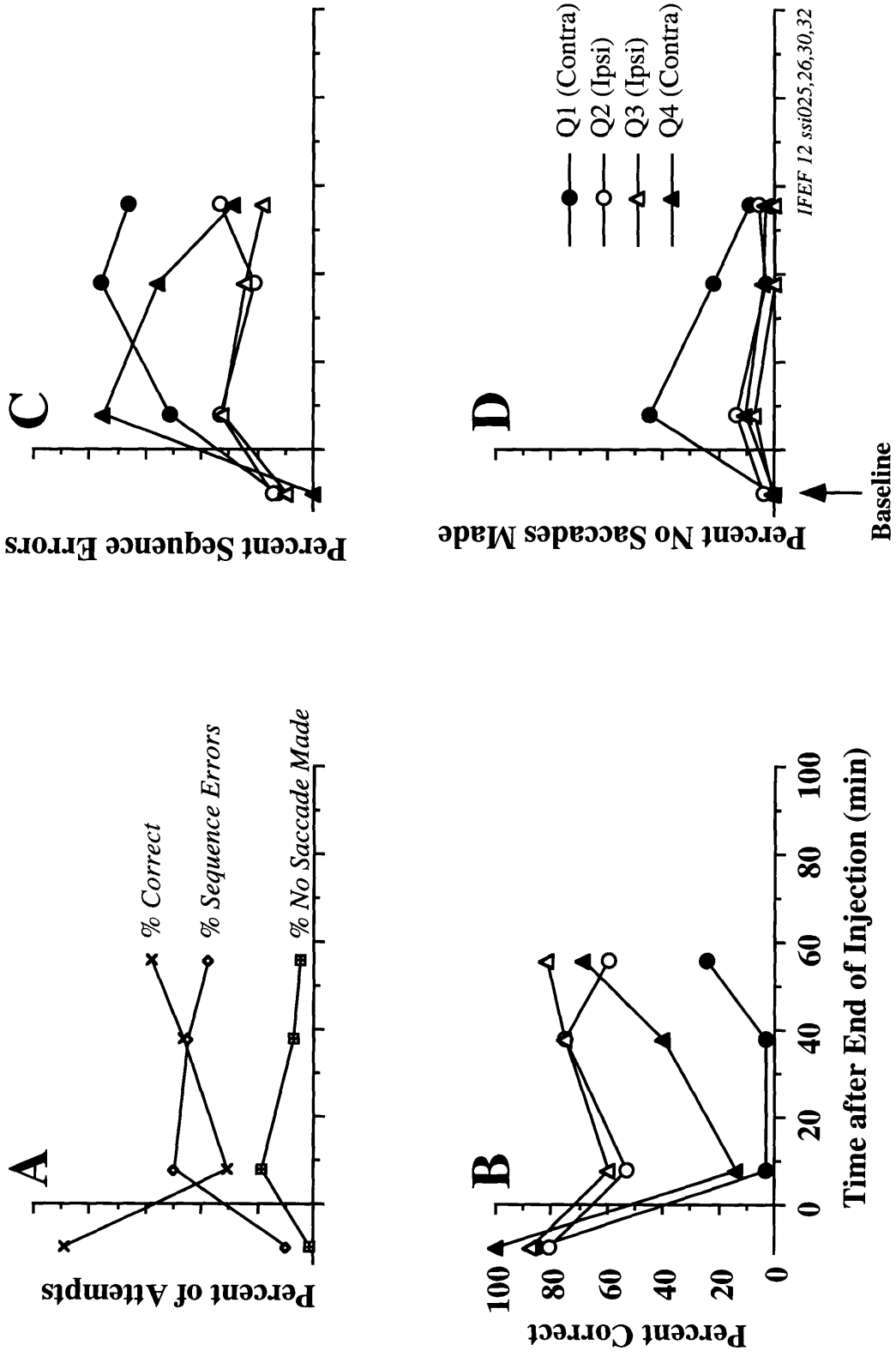
First saccades of the sequence, *Before* and *During* left FEF inactivation



T1 is in center of window,
T2 is in one of the other quadrants

Figure 17

Figure 18


A
% Correct
% Sequence Errors
% No Saccade Made
Percent of Attempts
B
Percent Correct
100
80
60
40
20
0
0
20
40
60
80
100
Time after End of Injection (min)
C
Percent Sequence Errors
D
Q1 (Contra)
Q2 (Ipsi)
Q3 (Ipsi)
Q4 (Contra)
IFEF 12 ssi025,26,30,32
Percent No Saccades Made
Baseline

Chapter 4

Summary and Conclusions

We examined the role of two regions of rhesus monkey frontal cortex, the FEF and DMFC, in the generation of saccades and fixations. We inactivated a FEF or DMFC with the anesthetic lidocaine while the monkey performed oculomotor tasks.

Reversible inactivation of FEF causes:

1. Nearly a complete loss of contraversive saccades made to remembered locations.
2. Nearly a complete loss of contraversive saccades made to brief visual flashes even if a memory period is not required. (Severity of these first two deficits was slightly modulated by eye position, for reasons that were unclear).
3. An increase in the latency of saccades made to all contralateral targets in all the tasks that we tested.
4. An increase in the frequency of premature saccades made to ipsilateral visual targets, stimuli only meant to provide a spatial cue during the memory task.
5. An increase in the failure rate of generating saccades at all to briefly flashed contralateral targets.
6. Quite often, deficits in the ability to initiate and maintain fixation at contralateral positions.
7. An ipsilateral shift of about 6 degrees in the dark.
8. An decrease in the peak velocity of 2 to 12 degree saccades (reason for this was unclear).

Many of these FEF inactivation results were confirmed to be due to grey matter inactivation by performing control injections of muscimol. Saline injections caused no effects.

Reversible inactivation of the DMFC causes:

1. None of the 8 effects listed above for FEF inactivation.
2. An impairment in the ability to generate two saccades in a sequence to sequentially flashed targets.

In conclusion, our results have provided new information on the role of frontal cortex in the generation of saccades and fixations. The FEF is involved in the direct generation of saccades and fixations, and it is especially necessary in difficult situations such as conditions of brief visual change (the "saw it out of the corner of my eye" phenomenon). The DMFC is not required for making saccades or fixations in simple tasks, but it is needed when saccades and fixations must be linked together to create a pattern of movement.

Although these results are encouraging, we are only beginning to understand how frontal cortex contributes to the coordination between visual perception and movements of the eyes. Looking at our surroundings in everyday life is so easy that we rarely appreciate the systems that have evolved to allow us such graceful, quick behaviors. It is this paradox, of the illusion of simplicity in our actions despite the known complexity of the neural systems that serve as effectors, that drives the curiosity of the neurophysiologist.

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