

# Overlap of Saccadic and Pursuit Eye Movement Systems in the Brain Stem Reticular Formation

YI-JUN YAN,<sup>1</sup> DONG-MEI CUI,<sup>1,2</sup> AND JAMES C. LYNCH<sup>1,2</sup>

<sup>1</sup>Department of Anatomy and <sup>2</sup>Department of Ophthalmology, The University of Mississippi Medical Center, Jackson, Mississippi 39216

Received 14 February 2001; accepted in final form 31 July 2001

**Yan, Yi-jun, Dong-mei Cui, and James C. Lynch.** Overlap of saccadic and pursuit eye movement systems in the brain stem reticular formation. *J Neurophysiol* 86: 3056–3060, 2001. Recent physiological studies have suggested that there are several sites of interaction between the neural pathways that control saccadic eye movements and those that control visual pursuit movements. To address the question of saccade/pursuit interaction from a neuroanatomical point of view, we have studied the connections from the smooth and saccadic eye movement subregions of the frontal eye field (FEFsem and FEFsac, respectively) to the rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF) in four *Cebus apella* monkeys. The riMLF has traditionally been considered to be a premotor center for vertical saccadic eye movements on the basis of single neuron recording experiments, microstimulation experiments, and surgical or chemical lesion experiments. We localized the functional subregions of the FEF with the use of low-threshold ( $\leq 50 \mu\text{A}$ ) intracortical microstimulation. Biotinylated dextran amine or lectin from triticum vulgaris (wheat germ), peroxidase labeled, was placed into these functionally defined subregions to label anterogradely the terminals of axons that originated in the FEF. Our results demonstrate that both the FEFsem and FEFsac send direct projections to the ipsilateral riMLF. The distribution and density of labeling from the FEFsem are comparable to those from the FEFsac. The direct FEFsem-to-riMLF projection suggests a possible role of the riMLF in smooth pursuit eye movements and supports the hypothesis that there is interaction between the saccadic and pursuit subsystems at the brain stem level.

## INTRODUCTION

The rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF) is a premotor center that has been traditionally associated only with the saccadic eye movement system (see Leigh and Zee 1999 for review). It contains neurons that display burst activity before vertical saccades (Büttner et al. 1977; Büttner-Ennever and Büttner 1978; King and Fuchs 1979). Inactivation or destruction of the riMLF produces defects in vertical rapid eye movements in monkeys (Crawford and Vilis 1992; Kompf et al. 1979; Suzuki et al. 1995) and in humans (Büttner-Ennever et al. 1982). Although much evidence supports a role in the saccadic system for the riMLF, some recent studies suggest that there is a functional interaction between the saccadic and pursuit eye movement subsystems at the level of the brain stem and cerebellum (Büttner-

Ennever et al. 1982; Kompf et al. 1979; Krauzlis and Miles 1998; Krauzlis and Stone 1999; Krauzlis et al. 1997, 2000; Missal et al. 1996, 2000; Takagi et al. 2000).

The riMLF projects to the oculomotor nucleus (Büttner-Ennever and Büttner 1978; Moschovakis et al. 1991a,b) and receives input from the paramedian pontine reticular formation (PPRF) (Büttner-Ennever and Büttner 1978), superior colliculus (SC) (Harting et al. 1980), and frontal eye field (FEF) (Huerta et al. 1986; Leichnetz and Gonzalo-Ruiz 1996). Previous studies of the connections from the FEF to the riMLF did not distinguish between the saccade subregion (FEFsac) and the pursuit subregion (FEFsem). We have studied for the first time the efferent connections of the physiologically identified smooth-pursuit subregion of the FEF using anterograde tracers. Biotinylated dextran amine (BDA) was chosen because it provides excellent visualization of axon terminal regions and terminal boutons, thus making it easy to distinguish between fibers of passage and terminal endings. Horseradish peroxidase conjugated to wheat germ agglutinin (WGA-HRP) provided qualitative confirmation of the BDA findings. We observed direct projections from both the FEFsac and FEFsem to the riMLF, with partially overlapping axon terminal distributions. These results provide direct neuroanatomical evidence for the possible interaction between these two oculomotor subsystems in the brain stem oculomotor system. Some of these results have been reported in abstract form (Yan et al. 2000).

## METHODS

The FEFsac and FEFsem were localized with intracortical microstimulation in four *Cebus apella* monkeys. Experiments were performed under sterile conditions, following a protocol approved by the Institutional Animal Care and Use Committee (Tian and Lynch 1996a,b). Trains (100- to 500-ms duration, 300 Hz) of unipolar pulses ( $< 150 \mu\text{A}$ ) were delivered by glass-insulated platinum-iridium electrodes ( $Z = 2\text{--}4 \text{ M}\Omega$ ), positioned under visual guidance. Eye movements were viewed on a video monitor and recorded on videotape for later quantitative analysis. The velocity and duration of visually guided eye movements have previously been compared with the velocity and duration of electrically evoked saccades in the same monkey under Telazol anesthesia using a magnetic search coil system (Tian and Lynch 1995). Eye-movement parameters using the video monitoring equipment were then compared with those using the

Address for reprint requests: Y. Yan, Dept. of Anatomy, The University of Mississippi Medical Center, 2500 N. State St., Jackson, MS 39216 (E-mail: jlync@anatomy.umsmed.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

TABLE 1. Summary table of tracer placements

Monkey	Tracer	Subregion of FEF
C21		
Left	10% BDA	Saccade
Right	2% WGA-HRP	Pursuit
C22		
Left	10% BDA	Pursuit
Right	10% WGA-HRP	Pursuit
C23		
Left	10% BDA	Pursuit
Right	10% WGA-HRP	Saccade
C26		
Left	10% BDA	Pursuit

search coil (Tian and Lynch 1995, 1996b). The video monitoring technique was found to be adequate to reliably differentiate between saccadic and pursuit-like movements. In most cases, tracer injections were restricted to cortical regions in which current levels  $\leq 50 \mu\text{A}$  elicited eye movements. The distributions of retrogradely labeled neurons in the thalamus were compared with those of previous experiments as a further verification that the FEFsem injections in these experiments were comparable to those reported in Tian and Lynch (1997). Most electrode placements were photographed through an operating microscope using either 35-mm film or a digital camera to aid in reconstructions of the stimulation sites. The direction of electrically evoked eye movements at injection sites within the FEFsem ranged from vertical to diagonal, usually with a predominant vertical component; the directions at injection sites within the FEFsac ranged from vertical to horizontal.

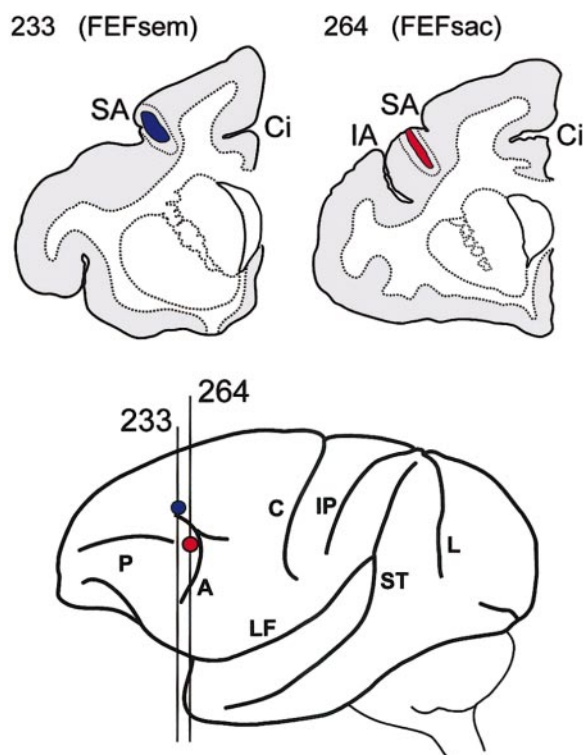


FIG. 1. Top: injection sites in the smooth eye-movement subregion of the frontal eye field (FEFsem) of monkey C23 (left) and in the saccadic eye-movement subregion of the FEF (FEFsac) of monkey C21 (right) in coronal sections. Single placements of biotinylated dextran amine (BDA) are within the gray matter of each functional subregion of the FEF. Bottom: a lateral view of the left hemisphere of monkey C23. The injection sites are marked by red (FEFsac) and blue (FEFsem) dots. The vertical lines indicate the cutting planes in the above sections.

After the functional subregions were defined, the anterograde tracers were delivered. The tracers used were BDA, 10,000 MW, lysine fixable (Molecular Probes), and WGA-HRP (Sigma). The BDA and WGA-HRP were used as 10% solutions in distilled water. Approximately  $0.6 \mu\text{l}$  of each tracer was pressure-injected at each site using a 1.0- or  $5.0\text{-}\mu\text{l}$  Hamilton syringe. Table 1 summarizes the animal cases for this study. Typical injection sites are illustrated in Fig. 1. After survival of 14–17 days, monkeys were deeply anesthetized and perfused transcardially with saline followed by mixed aldehyde fixative. Brains were blocked coronally and stored for 3 days in sucrose buffer, then frozen and cut at  $50 \mu\text{m}$ . One series of sections at  $300\text{-}\mu\text{m}$  intervals was stained for cytoarchitecture. Two series of sections adjacent to the cytoarchitecture sections were reacted for BDA and WGA-HRP, respectively. We used standard procedures to process BDA, using diaminobenzidine (DAB) as chromogen enhanced with nickel and cobalt (Liu and Mihailoff 1999; May et al. 1997; Veenman et al. 1992). For the HRP procedure, tetramethyl benzidine was used as chromogen, and ammonium molybdate was used as the stabilizing agent following the modified protocol of Mesulam (1978).

The BDA terminals were observed using a light microscope (Leitz DMR); the WGA-HRP labeled terminals were observed using polarizing filters. A digital camera (SPOT) on the microscope was used to capture cytoarchitecture images using a  $\times 1.6$  objective. Images of labeled terminals were captured with higher-power objectives. The location and relative density of BDA-labeled terminals was indicated on the cytoarchitecture images using CorelDraw.

## RESULTS

The riMLF consists of regularly spaced medium-sized multipolar cells. It is wing-shaped at its rostral pole, extends  $\sim 2$  mm medial-to-lateral, and characteristically has a large blood vessel outlining the dorsal margin (Figs. 2 and 3A). It extends  $\sim 2.5$  mm caudally to the point where the tractus retroflexus passes close to the nucleus of Darkschewitsch. Laterally, the cells become widely scattered, extending  $\leq 4$  mm from the midline, and the borders of the cell group cannot be clearly

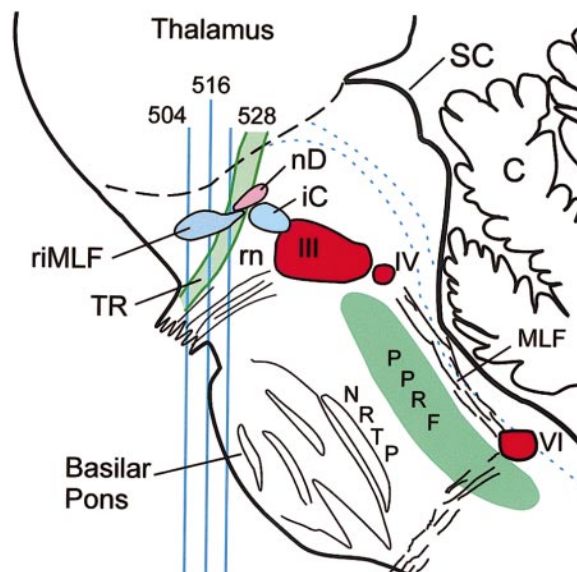


FIG. 2. Sagittal diagram of *Cebus* brain stem. The vertical lines indicate sections in monkey C21. iC, interstitial nucleus of Cajal; MLF, medial longitudinal fasciculus; nD, nucleus of Darkschewitsch; riMLF, rostral interstitial nucleus of the medial longitudinal fasciculus; rn, red nucleus; NRT, nucleus reticularis tegmenti; PPRF, paramedian pontine reticular formation; TR, tractus retroflexus; III, oculomotor nucleus; IV, trochlear nucleus; VI, abducens nucleus.



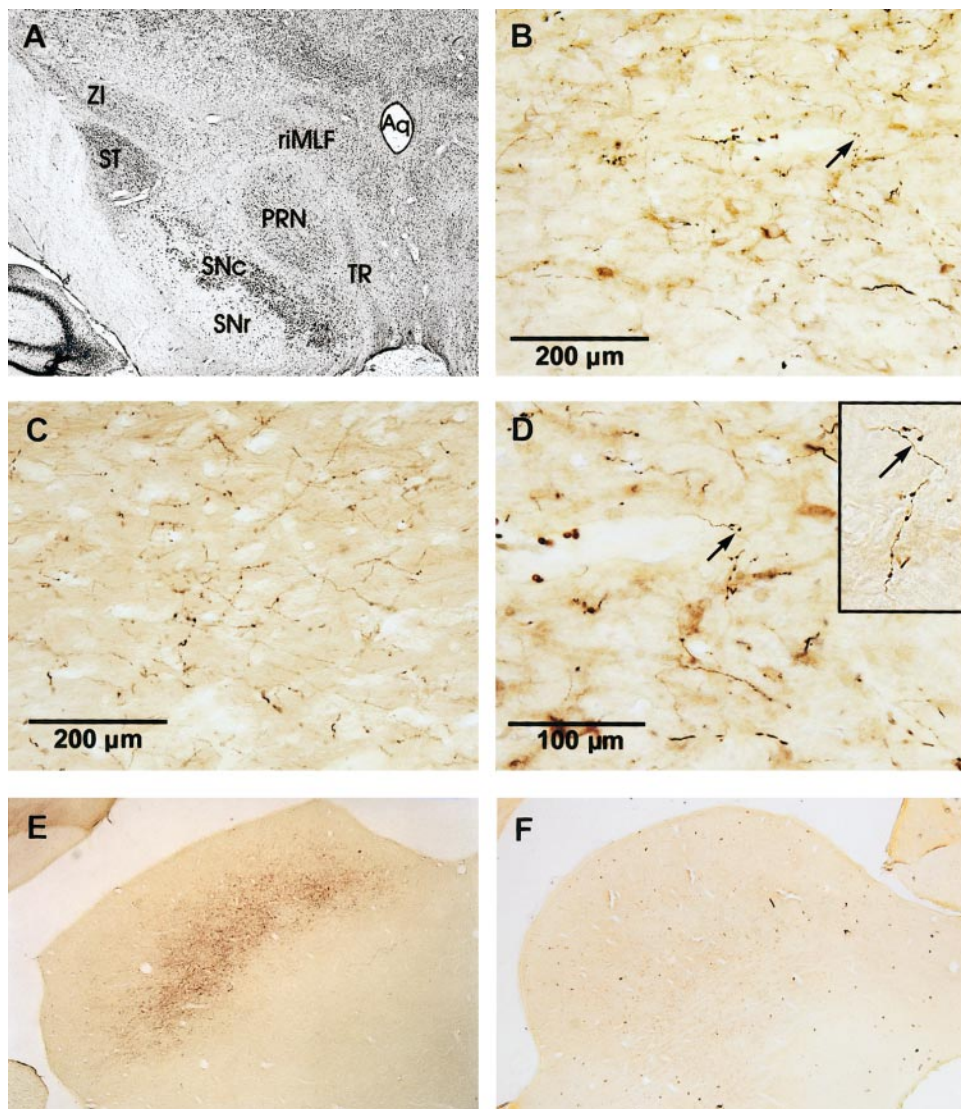


FIG. 3. Cytoarchitecture of rostral riMLF region (A) and labeling following injection into the FEFsac (C and E) and FEFsem (B, D, and F). B: labeling in riMLF after FEFsem injection (B). C: labeling in riMLF after FEFsac injection (C). D: higher-power view of region marked by arrow in B. *Inset*: oil immersion detail of terminals indicated by arrows. E: labeling in superior colliculus (SC) after FEFsac injection in *monkey C21*. F: labeling in SC after FEFsem injection in *monkey C23*. PRN, parvocellular red nucleus; SNc, substantia nigra, pars compacta; SNr, substantia nigra, pars reticularis; ST, subthalamic nucleus; ZI, zona incerta. Aq, cerebral aqueduct.

separated from other adjacent structures (Fig. 3A) (see also Fig. 3 in Büttner-Ennever and Büttner 1978).

The distribution patterns of labeled terminals from the WGA-HRP and BDA injections in a given region are similar. However, the BDA labeled terminals show more detail under higher objectives than those from WGA-HRP. We only include illustrations from BDA cases in this report. Typical terminal labeling after a BDA placement in FEFsac is illustrated in Fig. 3C (location indicated by dotted rectangle C in Fig. 4, *left*). Multiple terminal boutons are clearly visible. Typical labeling in the riMLF after a BDA injection in FEFsem is illustrated in Fig. 3, B and D (location indicated by dashed rectangle B in Fig. 4, *right*). The direct projections from the FEFsac to the SC are much stronger than the projections from the FEFsem to the SC (Fig. 3, E and F).

The distribution of BDA-labeled terminals in the region of the riMLF in two monkeys is illustrated in Fig. 4. The labeling from the FEFsac injection is located mainly in the medial portion of the riMLF (Fig. 4, *left*). There is also a small cluster just medial to the rostral part of the red nucleus, in agreement with Huerta et al. (1986). The labeling from the FEFsem injection is wing shaped and seems to fill a larger medial-

lateral extent of riMLF than that from the FEFsac injection (Fig. 4, *right*). The rostral-to-caudal extent of the labeling from the two subregions was similar. The distributions of labeling in C22, C24, and C26 were the same as in C23. This figure illustrates the two main results of this study. First, the FEFsem injections produced large distributions of labeled terminals within the anatomical boundaries of the riMLF, a structure previously supposed to be concerned only with saccadic eye movements. Second, within the riMLF there is partial overlap of the terminal distributions related to the injections in the saccadic and pursuit subregions of the FEF. The overlap is particularly evident in the second pair of sections (516 and 437). It should be noted that the FEFsac injection filled only a small fraction of the saccade subregion. If larger injections were made in the FEFsac, the area of terminal labeling in the riMLF and hence the region of overlap would be even larger (e.g., Fig. 3 in Huerta et al. 1986).

#### DISCUSSION

The present study is the first to describe direct projections from the physiologically-identified FEFsem to the riMLF. Pre-



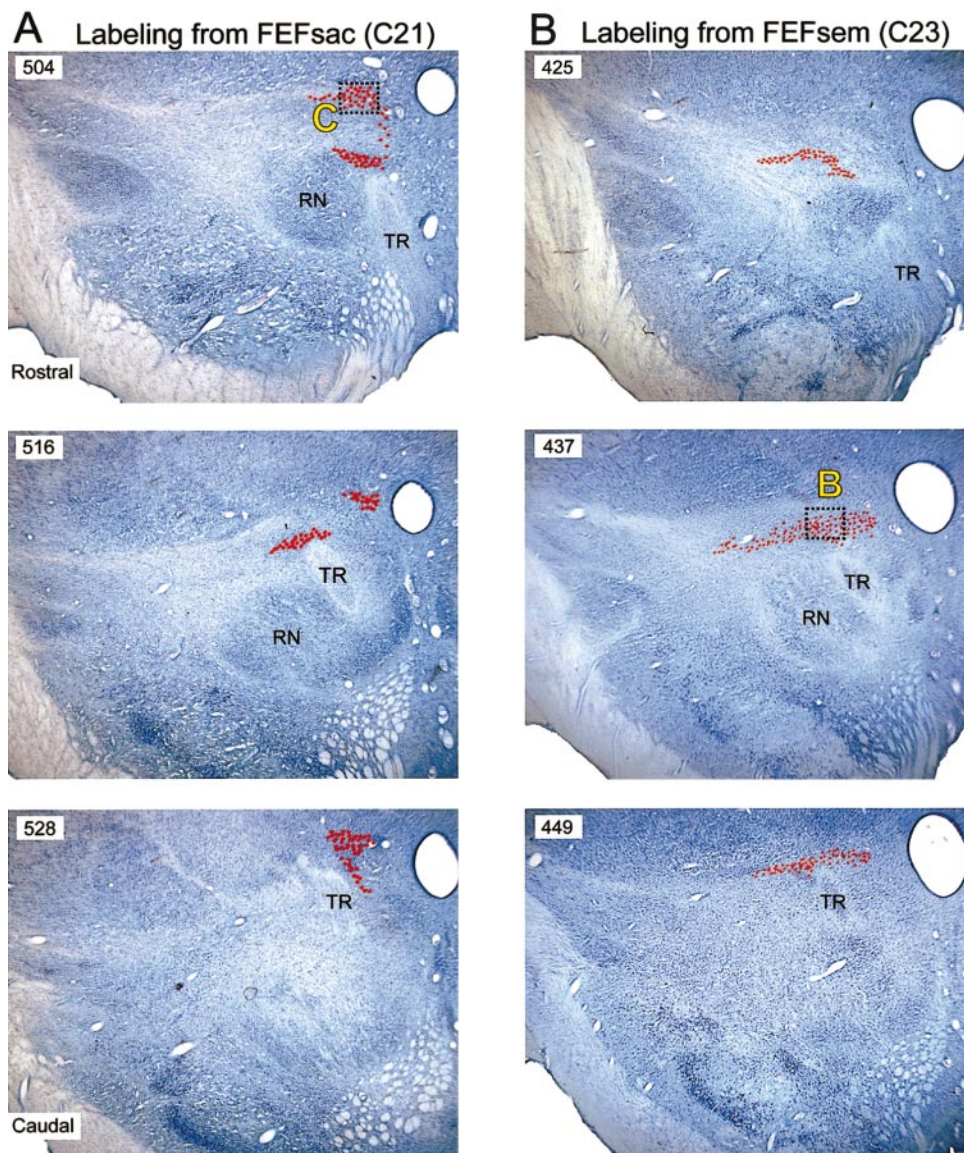


FIG. 4. The distribution and relative density of direct projections from the FEFsac (left) and FEFsem (right) to the riMLF. Sections are 600  $\mu$ m apart. The density of the labeling is represented by the relative density of the red dots. The dashed rectangle labeled C in section 504 indicates the position of the high-power photomicrograph illustrated in Fig. 3C. The dashed rectangle labeled B in section 437 indicates the position of the high-power photomicrograph in Fig. 3B.

vious studies of projections from the FEF did not discriminate between FEFsac and FEFsem (Huerta et al. 1986; Leichnetz and Gonzalo-Ruiz 1996). The FEFsem has been defined in *Cebus* as a small region on the dorsal bank of the superior tip of the arcuate sulcus. This cortical region is considered to be a distinct functional subregion because microstimulation there elicits pursuit-like eye movements; pursuit-related neural activity has been recorded there; and it is selectively connected to other cortical regions concerned with visual pursuit (Tian and Lynch 1996a,b, 1997). In addition, thalamo-cortical input to the FEFsem arises from different nuclei than does input to FEFsac (Tian and Lynch 1997) and FEFsac projects much more densely to the superior colliculus than does FEFsem (Fig. 3, E and F, present study). These observations make it unlikely that the terminal fields in the riMLF produced by FEFsem injections are the result of a small amount of functional overlap at the border between the FEFsem and FEFsac.

The distribution of labeled terminals from FEFsac to riMLF in the present study is in agreement with that reported by Huerta et al. (1986). This pathway may be part of a saccadic system that bypasses the SC and contributes to the rapid

recovery of saccadic eye movements that occurs after destruction of the SC (Schiller et al. 1980).

Neural signals related to pursuit eye movements have recently been observed in structures previously believed to be related only to saccadic eye movements. These include the interstitial nucleus of Cajal (iC) (Missal et al. 2000); superior colliculus (Basso et al. 2000; Krauzlis et al. 1997, 2000; Missal et al. 1996); cerebellar vermis (Suzuki and Keller 1988); and nucleus reticularis tegmenti pontis (NRTP) (Suzuki et al. 1999). Pursuit-like eye movements have been evoked by microstimulation in saccade-related structures including the cerebellar vermis (Krauzlis and Miles 1998; Takagi et al. 2000), superior colliculus (Missal et al. 1996), and NRTP (Yamada et al. 1996). Lesions in NRTP have produced deficits in visual pursuit (Suzuki et al. 1999). No recording or stimulation studies in riMLF have reported pursuit-related effects, but one clinical study reported pursuit deficits following restricted lesions in the region of the riMLF in humans (Büttner-Ennever et al. 1982).

Our results demonstrate that terminals labeled from a single small injection in the FEFsem are distributed from rostral to

caudal riMLF and from its medial region to its lateral-most extent. The density of terminals from the FEFsem is comparable to that from the FEFsac, and there is considerable overlap of the FEFsem and FEFsac terminal distributions. Signals from the FEFsac and FEFsem therefore converge on the riMLF, a structure that was previously considered to mediate only saccadic movements. These results suggest that the riMLF is one of a group of structures that are involved in the control and coordination of the conjoint saccadic and smooth pursuit eye movements needed to visually follow a moving object. Whether the direct projections to the riMLF from the two subregions of FEF actually terminate on single neurons cannot be answered by the present experiments. Our study does, however, provide the fundamental neuroanatomical basis for further functional studies.

We thank D. Holmes and J. Allison for technical assistance, P. May for histochemical advice, and M. King for helpful comments on the manuscript.

This work was supported by University Medical Center Intramural Research Support Program Grant 059918 and the Joe Weinberg Research Fund.

## REFERENCES

- BASSO MA, KRAUZLIS RJ, AND WURTZ RH. Activation and inactivation of rostral superior colliculus neurons during smooth-pursuit eye movements in monkeys. *J Neurophysiol* 84: 892–908, 2000.
- BÜTTNER U, BÜTTNER-ENNEVER JA, AND HENN V. Vertical eye movement related unit activity in the rostral mesencephalic reticular formation of the alert monkey. *Brain Res* 130: 239–252, 1977.
- BÜTTNER-ENNEVER JA AND BÜTTNER U. A cell group associated with vertical eye movements in the rostral mesencephalic reticular formation of the monkey. *Brain Res* 151: 31–47, 1978.
- BÜTTNER-ENNEVER JA, BÜTTNER U, COHEN B, AND BAUMGARTNER G. Vertical glaze paralysis and the rostral interstitial nucleus of the medial longitudinal fasciculus. *Brain* 105: 125–149, 1982.
- CRAWFORD JD AND VILIS T. Symmetry of oculomotor burst neuron coordinates about Listing's plane. *J Neurophysiol* 68: 432–448, 1992.
- HARTING JK, HUERTA MF, FRANKFURTER AJ, STROMINGER NL, AND ROYCE GJ. Ascending pathways from the monkey superior colliculus: an autoradiographic analysis. *J Comp Neurol* 192: 853–882, 1980.
- HUERTA MF, KRUBITZER LA, AND KAAS JH. Frontal eye field as defined by intracortical microstimulation in squirrel monkeys, owl monkeys, and macaque monkeys. I. Subcortical connections. *J Comp Neurol* 253: 415–439, 1986.
- KING WM AND FUCHS AF. Reticular control of vertical saccadic eye movements by mesencephalic burst neurons. *J Neurophysiol* 42: 861–876, 1979.
- KOMPF D, PASIK T, PASIK P, AND BENDER MB. Downward gaze in monkeys: stimulation and lesion studies. *Brain* 102: 527–558, 1979.
- KRAUZLIS RJ, BASSO MA, AND WURTZ RH. Shared motor error for multiple eye movements. *Science* 276: 1693–1695, 1997.
- KRAUZLIS RJ, BASSO MA, AND WURTZ RH. Discharge properties of neurons in the rostral superior colliculus of the monkey during smooth-pursuit eye movements. *J Neurophysiol* 84: 876–908, 2000.
- KRAUZLIS RJ AND MILES FA. Role of the oculomotor vermis in generating pursuit and saccades: effects of microstimulation. *J Neurophysiol* 80: 2046–2062, 1998.
- KRAUZLIS RJ AND STONE LS. Tracking with the mind's eye. *Trends Neurosci* 22: 544–550, 1999.
- LEICHNETZ GR AND GONZALO-RUIZ A. Prearcuate cortex in the Cebus monkey has cortical and subcortical connections like the macaque frontal eye field and projects to fastigial-recipient oculomotor-related brainstem nuclei [published erratum appears in *Brain Res Bull* 42: following III, 1997]. *Brain Res Bull* 41: 1–29, 1996.
- LEIGH RJ AND ZEE DS. *The Neurology of Eye Movements*. New York: Oxford Univ. Press, 1999, p. 104–105.
- LIU H AND MIHALOFF GA. Hypothalamopontine projections in the rat: anterograde axonal transport studies utilizing light and electron microscopy. *Anat Rec* 255: 428–451, 1999.
- MAY PJ, SUN W, AND HALL WC. Reciprocal connections between the zona incerta and the pretectum and superior colliculus of the cat. *Neuroscience* 77: 1091–1114, 1997.
- MESULAM MM. Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: a non-carcinogenic blue reaction product with superior sensitivity for visualizing neural afferents and efferents. *J Histochem Cytochem* 26: 106–117, 1978.
- MISSAL M, DE BROUWER S, LEFEVRE P, AND OLIVIER E. Activity of mesencephalic vertical burst neurons during saccades and smooth pursuit. *J Neurophysiol* 83: 2080–2092, 2000.
- MISSAL M, LEFEVRE P, DELINTE A, CROMMELINCK M, AND ROUCOUX A. Smooth eye movements evoked by electrical stimulation of the cat's superior colliculus. *Exp Brain Res* 107: 382–390, 1996.
- MOSCHOVAKIS AK, SCUDDER CA, AND HIGHSTEIN SM. Structure of the primate oculomotor burst generator. I. Medium-lead burst neurons with upward on-directions. *J Neurophysiol* 65: 203–217, 1991a.
- MOSCHOVAKIS AK, SCUDDER CA, HIGHSTEIN SM, AND WARREN JD. Structure of the primate oculomotor burst generator. II. Medium-lead burst neurons with downward on-directions. *J Neurophysiol* 65: 218–229, 1991b.
- SCHILLER PH, TRUE SD, AND CONWAY JL. Deficits in eye movements following frontal eye-field and superior colliculus ablations. *J Neurophysiol* 44: 1175–1189, 1980.
- SUZUKI DA AND KELLER EL. The role of the posterior vermis of monkey cerebellum in smooth-pursuit eye movement control. II. Target velocity-related Purkinje cell activity. *J Neurophysiol* 59: 19–40, 1988.
- SUZUKI DA, YAMADA T, HOEDEMA R, AND YEE RD. Smooth-pursuit eye-movement deficits with chemical lesions in macaque nucleus reticularis tegmentis pontis. *J Neurophysiol* 82: 1178–1186, 1999.
- SUZUKI Y, BÜTTNER-ENNEVER JA, STRAUMANN D, HEPP K, HESS BJ, AND HENN V. Deficits in torsional and vertical rapid eye movements and shift of Listing's plane after uni- and bilateral lesions of the rostral interstitial nucleus of the medial longitudinal fasciculus. *Exp Brain Res* 106: 215–232, 1995.
- TAKAGI M, ZEE DS, AND TAMARGO RJ. Effects of lesions of the oculomotor cerebellar vermis on eye movements in primate: smooth pursuit. *J Neurophysiol* 83: 2047–2062, 2000.
- TIAN JR AND LYNCH JC. Slow and saccadic eye movements evoked by microstimulation in the supplementary eye field of the cebus monkey. *J Neurophysiol* 74: 2204–2210, 1995.
- TIAN JR AND LYNCH JC. Functionally defined smooth and saccadic eye movement subregions in the frontal eye field of Cebus monkeys. *J Neurophysiol* 76: 2740–2753, 1996a.
- TIAN JR AND LYNCH JC. Corticocortical input to the smooth and saccadic eye movement subregions of the frontal eye field in Cebus monkeys. *J Neurophysiol* 76: 2754–2771, 1996b.
- TIAN JR AND LYNCH JC. Subcortical input to the smooth and saccadic eye movement subregions of the frontal eye field in Cebus monkey. *J Neurosci* 17: 9233–9247, 1997.
- VEENMAN CL, REINER A, AND HONIG MG. Biotinylated dextran amine as an anterograde tracer for single- and double-labeling studies. *J Neurosci Methods* 41: 239–254, 1992.
- YAMADA T, SUZUKI D, AND YEE R. Smooth pursuitlike eye movements evoked by microstimulation in macaque nucleus reticularis tegmenti pontis. *J Neurophysiol* 76: 3313–3323, 1996.
- YAN Y, CUI D, AND LYNCH JC. Pursuit subregion of the frontal eye field projects directly to brainstem premotor areas in Cebus monkey. *Soc Neurosci Abstr* 26: 1717, 2000.