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RESEARCH ARTICLE

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The feedback circuit connecting the superior colliculus and central mesencephalic reticular formation: a direct morphological demonstration

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Abstract The central mesencephalic reticular formation (cMRF) has been distinguished from the surrounding reticular formation due to its involvement in the control of saccades. A role in saccade function has been proposed for this region based on electrical-stimulation experiments, its neuronal activity, and its pattern of connections. The present study was undertaken in an attempt to further characterize the location of the central mesencephalic reticular formation by anatomical methods and to examine its connections with the superior colliculus at the neuronal level. Biotinylated dextran amine (BDA) was injected into the superior colliculus of two cynomolgus monkeys (*Macaca fascicularis*). This resulted in the retrograde labeling of a large number of neurons in a restricted area of the mesencephalic reticular formation. They were distributed bilaterally, with an ipsilateral predominance, forming a cellular band in the ventral half of the midbrain reticular formation that was 2.7 mm in its rostrocaudal extent. Its rostral pole lay dorsolateral to the red nucleus and ventrolateral to, but not immediately adjacent to, the interstitial nucleus of Cajal. The cell band was widest caudally, where it occupied an area of approximately 2.7 mm wide and 2 mm in depth. Labeled neurons displayed a wide variety of multipolar somatic shapes and sizes, with long, slightly tapering, sparsely branched dendrites. Tectal terminal arbors were also labeled within the mesencephalic reticular formation. They were concentrated bilaterally, with an ipsilateral predominance, in the same areas that contained retrogradely labeled neurons. Numerous, primarily en passant labeled boutons of various sizes and shapes were seen in close association with both labeled and unlabeled neurons. They formed axosomatic and, more commonly, axodendritic relationships with labeled neurons. The extensive relationship of labeled terminals and labeled cells suggests the existence of a strong interconnection between the deeper layers of the colliculus and the central mesencephalic reticular formation neurons projecting back to the tectum. The bidirectional neural circuit directly demonstrated in this study presumably provides an anatomical substrate for feedback modification of gaze signals generated in the colliculus. However, the presence of tectal terminals around unlabeled reticular neurons suggests that the collicular signal may also be fed forward to the downstream targets of the central mesencephalic reticular formation.

Key words Oculomotor · Primate · Saccade · Tectum · Gaze

Abbreviations *III* oculomotor nucleus · *VI* Abducens nucleus · *Aq* Cerebral aqueduct · *BC* Brachium conjunctivum · *BDA* Biotinylated dextran amine · *cMRF* Central mesencephalic reticular formation · *Cn* Cuneate nucleus · *HI* Habenulo-interpeduncular tract · *IC* Inferior colliculus · *IO* Inferior olive · *InC* Interstitial nucleus of Cajal · *LD* Lateral dorsal nucleus, thalamus · *LL* Lateral lemniscus · *MD* Medial dorsal nucleus, thalamus · *MGB* Medial geniculate body · *mlf* Medial longitudinal fasciculus · *nPC* Nucleus of posterior commissure · *PAG* Periaqueductal gray · *PC* Posterior commissure · *PRF* Pontine reticular formation · *Pul* Pulvinar · *Pt* Pretectum · *R* Red nucleus · *SGI* Stratum griseum intermediale · *SGP* Stratum griseum profundum · *SGS* Stratum griseum superficiale ·

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SMT Stria medullaris · *SN* Substantia nigra · *SO* Superior olive · *VL* Ventral lateral complex, thalamus · *VP* Ventral posterior nucleus, thalamus · *VS* Spinal trigeminal nucleus

Introduction

Due to its involvement in the control of saccades, it is possible to distinguish the central mesencephalic reticular formation (cMRF) from the surrounding reticular formation (Cohen and Büttner-Ennever 1984; Cohen et al. 1986). Evidence for its saccade-related function has come from studies that employ a variety of techniques. For example, lesions placed in this area in the monkey reticular formation cause transient deficits in gaze movements towards the contralateral side (Bender and Shanzer 1964; Komatsuzaki et al. 1972). In addition, electrical stimulation of this region induces contralateral saccadic eye movements in both cats (Szentagothai 1943) and monkeys (Bender and Shanzer 1964; Cohen and Büttner-Ennever 1984; Waitzman et al. 1996). The participation of the cMRF in saccade control gains further support from single-unit and intracellular-recording studies (Cohen et al. 1986; Moschovakis et al. 1988b; Waitzman et al. 1996; Handel and Glimcher 1997). These studies demonstrate that the neuronal activity of cMRF neurons precedes and codes for the horizontal components of contraversive saccades. Some more recent reports have implicated the cMRF in the generation of the vertical component of saccades as well (Handel and Glimcher 1997; Waitzman et al. 1997).

Like most reticular formation regions, the cMRF lacks distinct cytoarchitectonic boundaries. Its location has primarily been delineated by stimulation studies in monkeys (Cohen and Büttner-Ennever 1984; Cohen et al. 1986). These studies revealed that contraversive saccades could be evoked in only a restricted region of the mesencephalic reticular formation (MRF) that overlaps the rostral portion of the nucleus subcuneiformis in monkeys. However, in some, but not all reports, the cMRF has been described as extending lateral to the rostral interstitial nucleus of the medial longitudinal fasiculus (riMLF), in which case it would overlap with field H of Forel. These differences indicate that it may be useful to define the borders of the cMRF by anatomical means.

While there is little doubt that the cMRF participates in the control of gaze changes, its precise functional role in saccade circuitry has not been specified. Current hypotheses for the cMRF's functional role in the control of horizontal gaze changes emphasize its connections with the superior colliculus and the pontine reticular formation. In fact, anatomical studies in both monkeys (Harting 1977; Harting et al. 1980; Cohen and Büttner-Ennever 1984; Huerta and Harting 1984; Cohen et al. 1986; Moschovakis et al. 1988a, 1988b; Scudder et al. 1996) and cats (Graham 1977; Grantyn and Grantyn 1982; Grantyn et al. 1982) have provided evidence of tectal projections to the cMRF. In addition, projections from the cMRF to the intermediate and deep layers of the superior colliculus have also been demonstrated in monkeys (Moschovakis et al. 1988b) and cats (Edwards and de Olmos 1976; Grofova et al. 1978; Grantyn 1988; Appell and Behan 1990). Finally, direct connections between the MRF and the pontine regions directing horizontal gaze have been reported (Edwards 1975; Edwards and de Olmos 1976; Cohen et al. 1986; Langer and Kaneko 1984, 1990).

The interconnections between the superior colliculus and cMRF indicated above presumably provide an anatomical basis for the cMRF's functional role. However, the morphological details of this interconnection have not, to our knowledge, been described at a neuronal, as opposed to a regional, level. For example, it is not known whether the same cMRF cells that receive collicular inputs also project back to the superior colliculus. It is possible the tectal input is concentrated on cMRF neurons that project caudally to the pontine gaze centers. The nature of this connection has important implications for the role of the cMRF in gaze control. Thus, the present study in macaques attempts to address these questions by use of contemporary tracer methods capable of revealing neuronal and axonal morphology. This study also attempts to further characterize the location of the cMRF through an anatomical approach. Results from this study should provide a better anatomical basis for understanding the function of the central mesencephalic reticular formation.

Materials and methods

All animal procedures used in this study were undertaken in accordance with the animal care and use guidelines of the NIH and with the approval of the University of Mississippi Medical Center animal care and use committee. Two cynomolgus monkeys (*Macaca fascicularis*) underwent surgeries performed with sterile techniques. The animals were preanesthetized with ketamine hydrochloride (i.m.) and were maintained at a surgical level of anesthesia by ventilation with isoflurane. Supplemental fluids were given i.v., and core body temperature and vital signs were monitored and maintained within normal range. Animals were placed in a stereotaxic head frame and, after entering the skull, the portion of the medial occipital and parietal lobes overlying the midbrain was aspirated to allow direct visualization of the tectal surface. The tip of a 5-µl Hamilton syringe attached to a micromanipulator was centered in the intermediate layer of the superior colliculus, based on depth from the surface, and was used to inject a solution of biotinylated dextran amine (BDA). The colliculus on one side received multiple injections, with each injection consisting 0.1 µl of 10% BDA (total of 0.2 or 0.4 µl). The incision was closed after injection of the tracer. Buprenex (0.01 mg/kg, i.m.) was administered as an analgesic over the 24-h post-surgical recovery. The animals were carefully monitored during the 48 h following surgery. They behaved normally and showed no signs of distress during the survival period.

Following a survival period of approximately 2 weeks, monkeys were deeply anesthetized with sodium pentobarbital (70 mg/kg, i.p.) and perfused transcardially with buffered saline, followed by a fixative containing 1% paraformaldehyde and 1.25% glutaradehyde. The brain was blocked in the stereotaxic frontal plane, post-fixed for 1 h, and stored in cold 0.1 M (pH 7.2) phosphate buffer overnight. Frontal sections through the MRF

were cut at 100 µm in thickness with a vibratone. The sections were divided into three groups, with each group representing a series of 300 µm intervals. Two of these series were prepared for histochemical demonstration of biotin (Olivier et al. 1998). Sections were incubated for 12 h in avidin D conjugated to horseradish peroxidase (Vector, 1:5000) in 0.1 M (pH 7.2) phosphate buffer containing 0.05% Triton X-100. After rinsing in phosphate buffer several times, sections were then preincubated in 0.05% diaminobenzidine (DAB) solution in 0.1 M (pH 7.2) phosphate buffer that contained 0.001% cobalt chloride and nickel ammonium sulfate. Addition of 0.003% hydrogen peroxide was made to reveal the reaction product. Sections were then mounted, counterstained with cresyl violet, dehydrated, cleared, and coverslipped. By use of a photomicroscope equipped with a drawing tube, the labeled profiles in all the mesencephalic reticular formation sections were inspected at a magnification of ×100 and charted at 600-µm intervals. Individual cells and arbors were drawn and photographed at a magnification of ×600–1000.

Results

Figure 1A, B shows a case in which multiple injections resulted in tracer infiltration throughout a large extent of the superior colliculus. While tracer was centered in the intermediate gray layer of the colliculus, the deeper layers were also markedly invaded. Except for slight encroachment on the pretectum (A) and dorsal part of periaqueductal gray, tracer spread to other adjacent structures was not obvious. The other case (Fig. 1C–E) had a smaller injection that was restricted to the central portion of the colliculus and did not spread outside its borders. Similar results were observed in these two cases, although more limited labeling was seen in the case with the smaller injection.

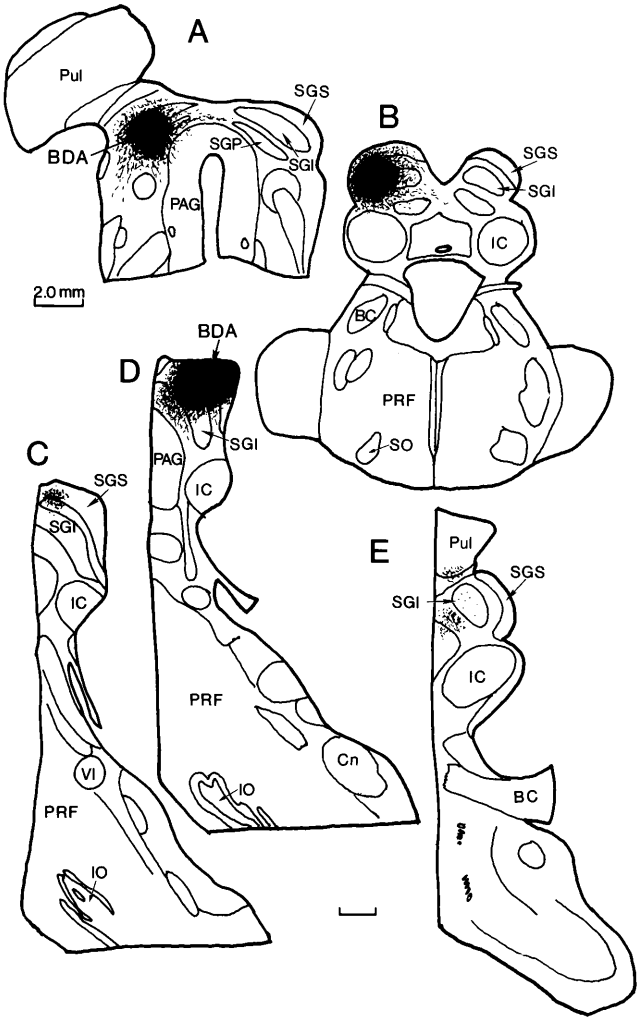


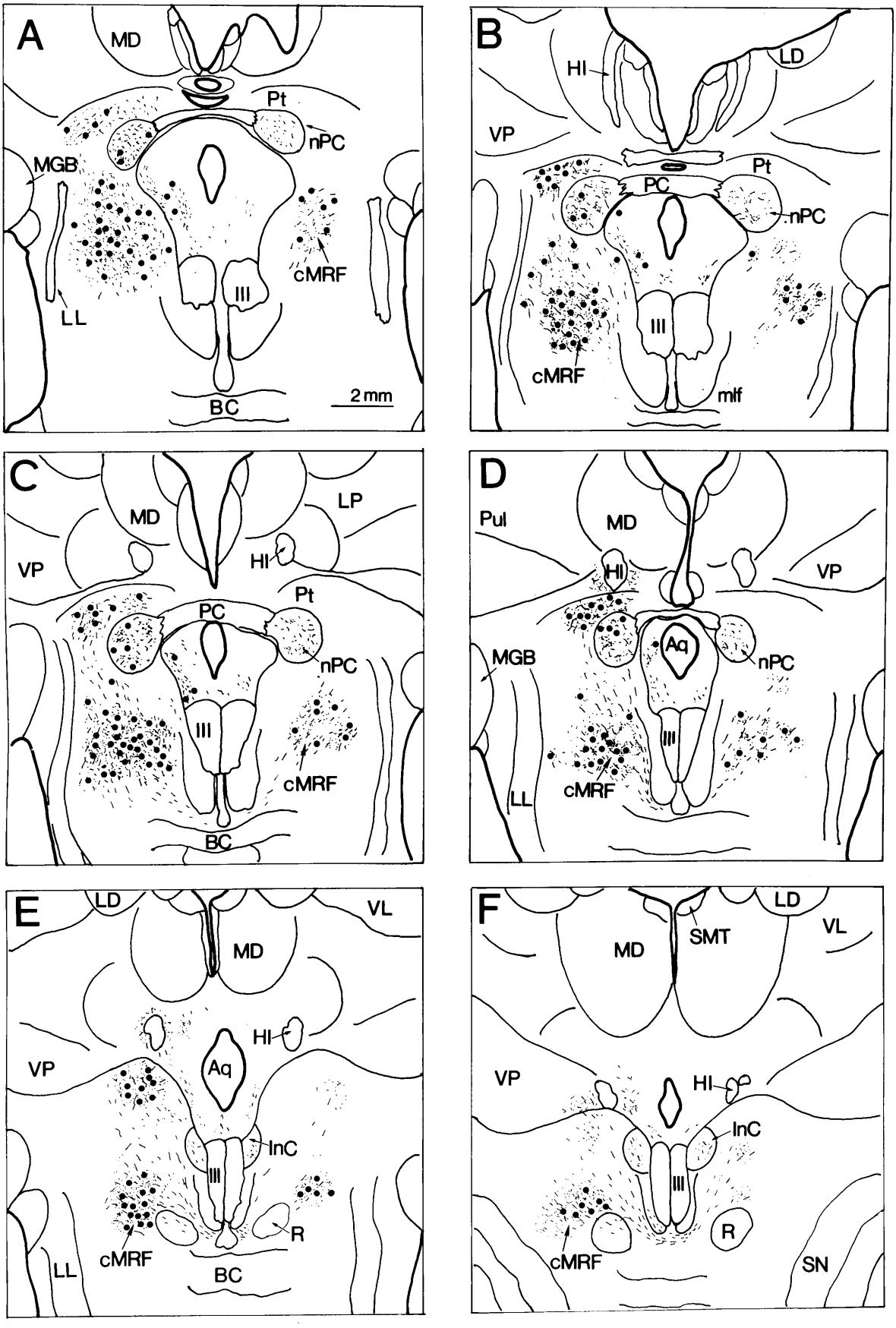
Fig. 1A–E Schematic drawings showing unilateral tracer (*BDA* biotinylated dextran amine) injection sites in the superior colliculus of two macaques. In the first case, **A** is rostral to **B**. In the second case, sagittal section **C** is medial and **E** lateral. *Dark areas* represent the core of the injection site, where the reaction product was the dominant element, and *stippled areas* represent the region of lighter tracer spread

Distribution of labeled tectal terminal fields

Figure 2 shows the pattern of labeling following the injection illustrated in Fig. 1A, B. Numerous labeled axons exited the injection site in the superior colliculus and aggregated into several pathways with different trajectories and destinations, as have been described previously (see Graham 1977; Harting 1977; Harting et al. 1980). Among these, a labeled fiber pathway was observed to travel ventrally into the mesencephalic reticular formation (MRF) (Fig. 2A–F). This pathway consisted of a large number of labeled axons (lines) of various diameters and orientations. The axons were distributed in areas lateral to the periaqueductal gray and oculomotor nucleus. In the ventral portion of the MRF, a portion of the thick axons aggregated into a compact fiber tract that crossed in the dorsal tegmental decussation (Fig. 2E, F) and descended as the predorsal bundle, lateral to the nucleus reticularis tegmenti pontis on the contralateral side (not illustrated). Before crossing the midline, labeled tectal axons appeared to ramify, giving rise to branches that terminated profusely within the substance of the MRF. Although present throughout the MRF, labeled tectal terminals (stipple) were not evenly distributed in the MRF. They were most prevalent in areas that contained retrogradely labeled neurons (dots) and numerous thick axons of passage that ran rostrocaudally. The contralateral MRF also contained a similar, but much less prominent pattern of labeled tectal terminals distributed around labeled neurons. Labeled terminal arbors were also observed bilaterally in the nucleus of the posterior commissure (Fig. 2A–D), field H of Forel including the riMLF (not shown), and, to a much lesser degree, in the interstitial nucleus of Cajal (Fig. 2E–K) and the immediately adjacent reticular formation.

Retrogradely labeled neurons

Figure 2 also illustrates the distribution of midbrain neurons that were retrogradely labeled following injections of BDA into the macaque superior colliculus. Two con-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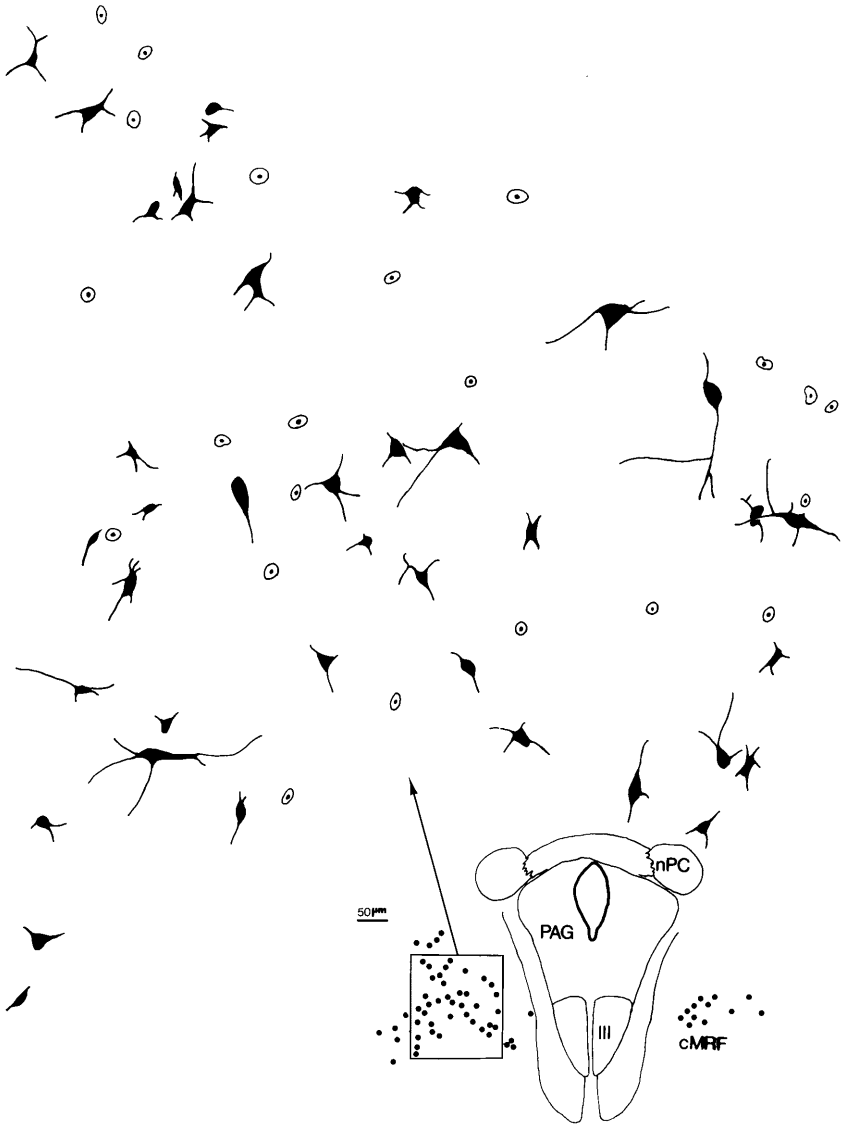
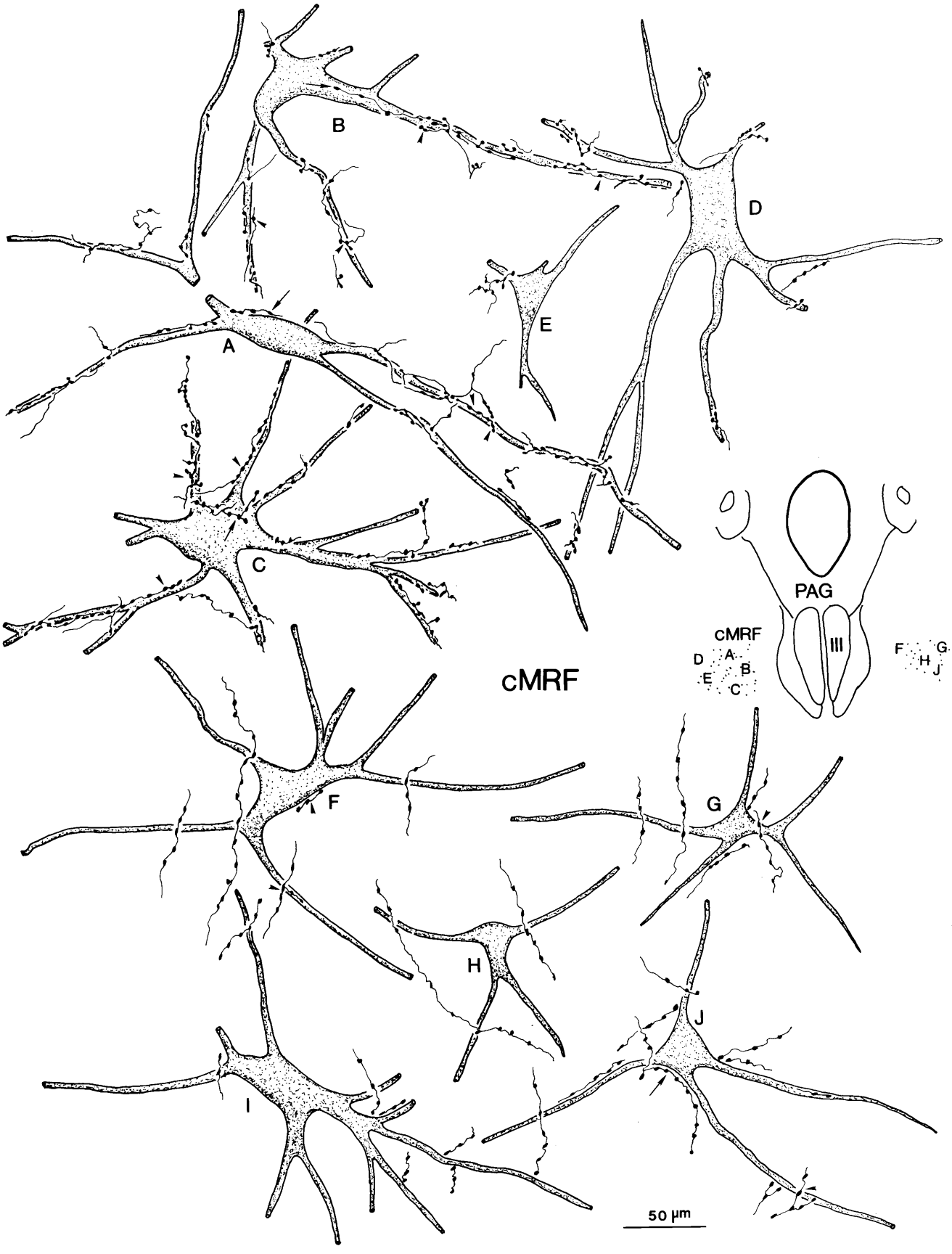


Fig. 3 The intermixed arrangement of labeled (*dark*) and unlabeled (*blank*) neurons in a single section through the ipsilateral MRF. The location of labeled cells is indicated in the lower right corner

centrations of retrogradely labeled neurons (dots) can be observed here. A dorsal population of labeled neurons (Fig. 2B–E) was located dorsal and caudal to the nucleus of the posterior commissure. These are presumably located in the pretectum and will not be discussed further. A ventral group of labeled neurons (Fig. 2A–F) was located in a restricted area corresponding to the dorsoventral location of the central mesencephalic reticular formation (cMRF). These labeled neurons were found in cMRF bilaterally, with an ipsilateral predominance. They were distributed throughout the MRF, lateral to the periaqueductal gray. A loose aggregation of labeled neurons first appeared at the level of the caudal oculomotor complex (Fig. 2A) and filled the dorsal half of the MRF. Just rostral to this level (Fig. 2B), the labeled cells became concentrated into a band that occupied an area approximately 2.7 mm wide and 2 mm deep. This band of labeled neurons shifted ventrally, relative to the oculomotor nucleus, as it extended rostrally (Fig. 2B–F). At the rostral end, the band narrowed, and labeled cells were located dorsal and lateral to the red nucleus and ventrolateral to the interstitial nucleus of Cajal (Fig. 2E–F). The whole rostrocaudal extent of this group of labeled neurons was approximately 2.7 mm. The oculomotor nucleus, extending 4.5 mm rostrocaudally in the midbrain, makes a convenient reference point. The distribution of the labeled cMRF neurons overlapped the caudal 60% (2.7 mm) of the oculomotor nucleus. We paid special attention to the interstitial nucleus of Cajal (InC) and the area immediately adjacent to it. While a few labeled tectal terminals were present there, almost no labeled neu-

◀ **Fig. 2A–F** Charting of the distribution of the retrogradely labeled neurons (*dots*) and labeled tectal axons (lines) and terminals (stipple) in the cMRF. Coronal sections are arranged in caudal (**A**) to rostral (**F**) order. Note that a concentration of labeled tectal terminals overlaps the area where labeled neurons are found. Labeled neurons and tectal terminals are fewer in number contralaterally. The *dots* represent individual cells present in the section charted. Due to their great number and small size, the *stipple* just indicates the relative concentration of terminals


B
D
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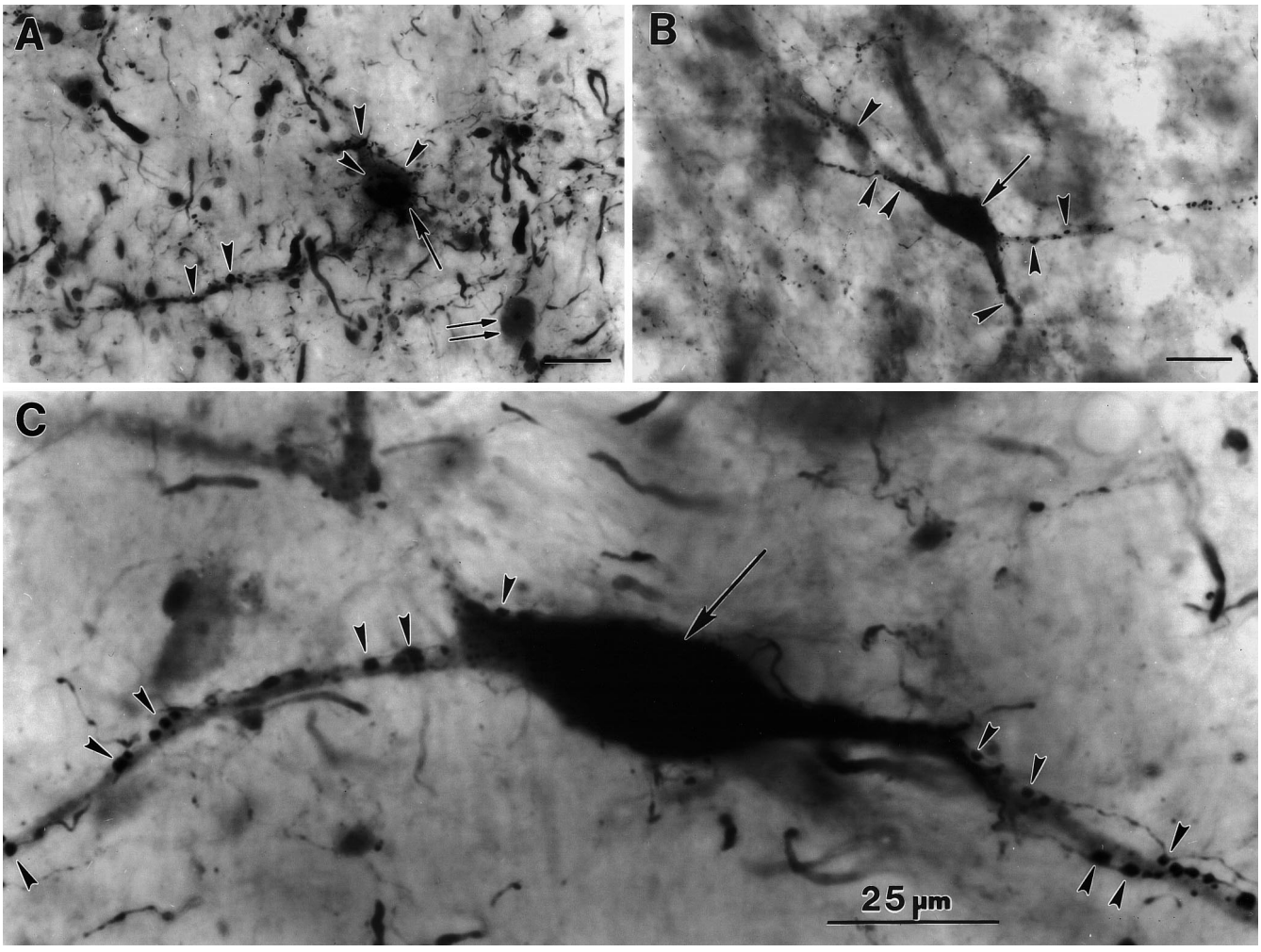

Fig. 5 Photomicrographs show the relationships between anterogradely labeled tectal axon arbors (*arrowheads*) and retrogradely labeled (**B, C** *arrows*) and unlabeled (**A** *arrow*) neurons in the ipsilateral (**A, C**) and contralateral (**B**) cMRF. Lightly stained BDA-labeled cells were chosen so that terminal appositions could be easily seen. BDA-labeled tectal boutons form numerous appositions (*arrowheads*) with the dendrites of the BDA-labeled (**B, C**) and counterstained (**A**) neurons. However, not all cells received such dense terminations (**A** *double arrows*). Note the greater density of labeled tectal axons in the ipsilateral cMRF neuropil (**A, C**) compared with that of the contralateral cMRF (**B**)

rons were found in this region. An additional population of small labeled cells overlapped by a terminal field was seen in the MRF rostral to the area illustrated. However, these were located at the dorsal border of the MRF, and so were not considered to be part of the cMRF.

◀ **Fig. 4** Drawings demonstrate the morphology of representative BDA-labeled neurons and their relationships with the BDA-labeled tectal terminals in the cMRF ipsilateral (cells **A–E**) and contralateral (cells **F–J**) to the tectal injection. Locations of labeled cells are indicated in the *right hand panel*. Boutons on the labeled collicular axons form both axosomatic (*arrows*) and axodendritic (*arrowheads*) relationships with labeled cMRF neurons

Figure 3 illustrates labeled and unlabeled neurons in the cMRF in a single coronal section. Retrogradely labeled cells (filled) were observed to distribute randomly within this cell group and were intermixed with unlabeled neurons (outlined). This suggests that tectal efferents are intermixed with other cMRF neurons, presumably interneurons and/or efferents projecting to non-tectal targets. The labeled cells usually had multipolar somata, and they displayed a wide variety of shapes and sizes. There was no apparent segregation of neurons according to size or shape. The somata of the labeled neurons could be as large as 83×33 µm (long × short axes) or as small as 10×8.3 µm. The average size for this group of labeled neurons was 28×15 µm (long axis SD = 4.07 µm; short axis SD = 1.58 µm; n=100). A higher magnification view of selected, well-labeled ipsilateral (A–E) and contralateral (F–J) reticulotectal neurons is provided in Fig. 4. Most labeled neurons had 3–7 long, thick primary dendrites radiating from the somata with apparently random orientations. These dendrites had little taper and could be followed over 200 µm through the neuropil in a single 100 µm section. Within the extent of the observable dendritic field, secondary dendrites were uncommon and were usually derived from primary dendrites near the somata. Dendritic spines

or varicosities were not observed. There was no obvious morphological difference between labeled neurons in the ipsilateral cMRF (Fig. 4A–E) and contralateral cMRF (Fig. 4F–J).

Relationship between tectal terminals and cMRF neurons

As was shown in Fig. 2, the distribution of the anterogradely labeled tectal terminal field overlapped that of the retrogradely labeled neurons in the cMRF region. Fewer terminals were present rostral, dorsal, and ventral to the band of labeled cells, although this difference was less pronounced caudally. The neuronal relationship between these two labeled elements is demonstrated in Fig. 4. Large numbers of preterminal and terminal fibers were observed in the cMRF region, and they displayed numerous en passant and terminal swellings. These presumed synaptic boutons were of spherical or ellipsoid shape, and they varied in size and shape, even on an individual fiber. Many of these labeled tectal terminals formed either axodendritic or axosomatic associations with labeled neurons both ipsilateral (Fig. 4A–E) and contralateral (Fig. 4F–J) to the BDA injection. However, axodendritic relationships were encountered much more often. In fact, tectal axons possessing many en passant boutons were often seen running immediately beside the dendrites of labeled neurons (Fig. 4A, B, and J). These presumptive terminals suggest a significant synaptic influence over these labeled neurons.

This pattern of an axon following a labeled dendrite is further demonstrated in the photomicrographs in Fig. 5. The BDA reaction product labeled the dendritic processes of a cell in the ipsilateral cMRF (Fig. 5C). Labeled axons with en passant swellings (arrowheads) can be observed following along the course of these dendrites. The close association between tectal terminals and cMRF dendrites is apparent in this higher magnification view. Although fewer in number due to the more limited labeling contralaterally, close associations between the labeled tectal axon terminals and labeled neurons were also observed in the contralateral cMRF (Fig. 5B). Tectal terminals were sometimes found to be in close association with the somata and the initial portion of the proximal dendrites belonging to unlabeled neurons in the cMRF as well (Fig. 5A). There were, of course, many terminal ramifications in the neuropil on both sides that could not be directly related to either labeled or unlabeled neurons. Furthermore, not all of the unlabeled cells had labeled terminals associated with them (Fig. 5A), and there were differences in the extent to which labeled terminals were associated with labeled reticulotectal cells as well.

Discussion

The results of this study indicate that the central region of the mesencephalic reticular formation is characterized by an overlap between tectoreticular terminal arbors and reticulotectal cells. Furthermore, the connection appears to be a direct reciprocal one at the neuronal level, because the boutons on the terminal arbors are often closely associated with the reticulotectal cell dendrites. As with all tracer studies, the exact borders of the area of tracer uptake can not be determined. However, there appeared to be only slight spread of tracer outside the borders of the colliculus observed in the larger injection and none in the smaller one. This point was supported by analysis of the pattern of labeling, which did not indicate spread into adjacent structures, e.g., the inferior colliculus. It is possible that some of the labeled terminals observed in the MRF represent collaterals of tectal afferents, but, in light of the evidence from previous studies (Harting et al. 1980), the presence of a collicular projection to the MRF seems assured. It is also possible that some of these arbors represent recurrent collaterals, but local collaterals were not noted in intracellular investigations of reticulotectal cMRF cells (Moschovakis et al. 1988b).

Location of the central mesencephalic reticular formation

The location of the central mesencephalic reticular formation (cMRF) in the monkey has been primarily specified by stimulation studies (Cohen and Büttner-Ennever 1984; Cohen et al. 1986). Initially, the cMRF was defined as an area within the midbrain reticular formation approximately 2 mm wide, 1.5 mm deep, and 3 mm in rostrocaudal extent, where contraversive horizontal saccadic eye movements were elicited upon electrical stimulation. These saccades were still induced following chemical ablation of the ipsilateral colliculus (Cohen et al. 1986), indicating the stimulation effects were probably not caused by activation of the tectofugal fibers traveling through the cMRF (Edwards and de Olmos 1976; Harting 1977; Harting et al. 1980; Grantyn and Grantyn 1982; Moschovakis et al. 1988b). In subsequent studies, single-unit and intracellular-recording techniques indicated the presence of long-lead burst neurons with saccade-related activity in this region (Moschovakis et al. 1988b; Waitzman et al. 1996; Handel and Glimcher 1997).

Results from the present study indicate that a concentration of retrogradely labeled cMRF neurons and anterogradely labeled tectal terminations overlaps extensively in the central portion of MRF. This region of overlap (2.7 mm wide, 2 mm deep, 3 mm long) closely matches the extent and is found in the general location of the cMRF, as defined by microstimulation studies describing this saccade-related region (Cohen and Büttner-Ennever 1984; Cohen et al. 1986). Recently, Waitzman and colleagues (1996) provided a detailed reconstruction of the MRF region that contains cells coding for the horizontal components of saccades. The area of overlap between tectoreticular axons and reticulotectal cells shown

here (Fig. 2) lies within the borders of this physiologically defined cMRF. Like the anatomically defined region, it spreads dorsoventrally at its caudal end, but this physiologically defined cMRF appears to have a longer rostrocauldal extent (4 mm). Thus, we believe it may be possible to identify an anatomically defined cMRF as the region in the MRF in which there is extensive overlap of collicular afferents and efferents. Experiments that directly correlate physiological and anatomical techniques would strengthen this hypothesis. Nevertheless, this study provides an additional tool with which the cMRF can be examined.

The anatomically defined cMRF does not occupy a uniform area along its rostrocaudal extent. Caudally, the border is somewhat indistinct, where labeled cells are not concentrated in a band (Fig. 2A). At the rostral end, it becomes smaller and takes a more ventral position. There, the band of labeled cells observed in this study lies ventrolateral to, but not immediately adjacent to the interstitial nucleus of Cajal (InC). It does not extend to the level of the rostral interstitial nucleus of medial longitudinal fasciculus (riMLF).

The fact that the anatomically defined cMRF does not extend lateral to the riMLF does not agree in this detail with an earlier study that indicated a more rostral extent for the cMRF (Cohen and Büttner-Ennever 1984). This discrepancy may be due to stimulus spread or electrical activation of cMRF afferent fibers traveling lateral to the riMLF. In fact, other physiological studies have shown that, in contrast to cMRF neuron activity, the areas in and lateral to the riMLF contain long-lead burst neurons primarily encoding the vertical components of gaze changes (Büttner-Ennever and Büttner 1978; Nakao et al. 1990; Shiraishi and Nakao 1994). While it is unlikely that the cMRF extends to the level of the riMLF(see also Waitzman et al. 1996), it is possible that the rostral pole of the physiologically defined cMRF does not contain reticulotectal neurons, although it may contain neurons that receive tectal input and display activity related to the horizontal components of saccades. Specifically, comparison of the present data to the plots in Cohen and Büttner-Ennever (1984), Cohen et al. (1986), and Waitzman et al. (1996) suggests that the physiologically defined cMRF may extend slightly rostral to the anatomically defined cMRF demonstrated here. If true, this would suggest that there may be functional subdivisions within the cMRF. On the other hand, this difference may reflect the vagaries of interspecies differences or intraspecies cranial variations producing plane-of-section effects, which are particularly acute at the level of the midbrain flexure. Curiously, the identified reticulotectal cells labeled in the squirrel monkey appear to be located caudal and dorsal to those observed in the present study (Moschovakis et al. 1988b). This may be a species difference, or it may represent the effects of cutting the brain in a different plane and collapsing the reconstruction onto a single section.

Recently, Handel and Glimcher (1997) proposed that cMRF neurons can discharge in relation to both the horizontal and vertical components of gaze changes. Thus, the precise physiological definition of the cMRF is still problematic. The recording sites illustrated by Handel and Glimcher (1997) are located in the portion of the midbrain ventrolateral to the InC. Waitzman and colleagues (1997) have shown that vertical eye movements are impaired following chemoinactivation of sites in the MRF closer to the interstitial nucleus of Cajal. This location correlates with the rostral end of our distribution of labeled cMRF neurons (Fig. 2F). However, most of the cMRF reticulotectal cells are distributed more caudally, at the level of the posterior commissure. Reticular, long-lead burst neurons, which discharge with regard to the horizontal amplitude of contraversive saccades, are found at the level of the posterior commissure (Waitzman et al. 1996), and microstimulation here produces contraversive horizontal saccades (Cohen et al. 1986; Fig. 1). Perhaps the rostromedial regions of the MRF contain the vertical components of this gaze center, although stimulation at more rostral levels still produces primarily horizontal saccades (Cohen and Büttner-Ennever 1984; Cohen et al. 1986).

Certainly, neurons located caudolaterally in the MRF differ in their firing properties from vertical gaze cells in and immediately adjacent to the InC (Fukushima et al. 1995; Kaneko and Fukushima 1998). A distinction between these two regions also gains support from the present data and other anatomical studies. For example, by injecting HRP into the physiologically identified omnipause region in the cat, Langer and Kaneko (1984) found that, while large numbers of retrogradely labeled neurons were present in nucleus cuneiformis, almost no labeled neurons were seen in and immediately adjacent to the InC. We conclude that the anatomically defined cMRF shown here lies within the region believed to encode the horizontal components of saccades, as the cMRF was originally defined. Furthermore, it includes the area where Handel and Glimcher (1997) observed neurons coding for both planes. It does not appear to overlap with the InC and immediately adjacent areas involved in producing vertical eye movements. We would argue that the terminology used to discuss these regions should eventually reflect these differences.

Neuron heterogeneity

The present study displays the morphological profiles of a wide variety of cMRF neurons that were retrogradely labeled from collicular tracer injections. These labeled neurons have multipolar perikarya of various sizes and shapes and are randomly distributed in the cMRF. As expected, the morphology of labeled cells demonstrated here resembles that of intracellularly investigated reticulotectal neurons discharging in relation to contraversive saccades and projecting exclusively to the superior colliculus (Moschovakis et al. 1988b).

Previous anatomical studies concerning cMRF connections with oculomotor-related structures imply that

cMRF contains at least two types of projection neurons based on axonal targets: neurons that project exclusively to the superior colliculus, as demonstrated by intra-axonal staining (Moschovakis et al. 1988b), and neurons that project to the pons (Edwards 1975; Edwards and de Olmos 1976; Büttner-Ennever and Büttner 1978, 1988; Huerta and Harting 1984; Langer and Kaneko 1984, 1990). Langer and Kaneko (1984, 1990) reported that a large number of neurons were retrogradely labeled in the MRF following HRP injections of the omnipause region in the cat and monkey. The general location of these labeled cells corresponds to that of the cMRF identified in the present study. Thus, the retrogradely labeled neurons displayed in the current study presumably correspond to cells projecting exclusively to the superior colliculus, and neurons that are not labeled may correspond to interneurons and/or neurons projecting to other areas, particularly the pons. There is some evidence that cMRF neurons may be heterogeneous in terms of their neurotransmitters. Studies in rat and cat suggest that a portion, but not all, of the tectally projecting neurons and those with other targets may be GABAergic (Araki et al. 1984; Appell and Behan 1990). There is physiologic evidence for heterogeneity as well. Neurons with different levels of background firing rate and different responses to saccade parameters have been recorded in the cMRF (Waitzman et al. 1996). These various transmitter-specific or physiological classes may be related to axon target or the variations in cell size and dendritic morphology observed in the present material.

Connections with the superior colliculus

The mesencephalic reticular formation has been shown to be a major target of collicular output (Harting et al. 1980; Cohen and Büttner-Ennever 1984; Huerta and Harting 1984). This projection appears to be derived from the collaterals of tecto-reticulo-spinal tract axons branching before they decussate into the contralateral predorsal bundle (Grantyn and Grantyn 1982; Moschovakis et al. 1988a, 1988b). Results from the present study confirm the presence of a collicular projection to the primate MRF. More importantly, this study directly displays the extensive overlap of anterogradely labeled tectal terminations and retrogradely labeled cMRF neurons. The intensity of tectal projections to the cMRF and the close apposition of multiple labeled tectal terminals to individual labeled and unlabeled cMRF neurons shown by the current results indicate that the superior colliculus has substantial influence over the neuronal activity in this region. Although ultrastructural analysis would be necessary to prove synaptic contacts are present, the light microscopic evidence is compelling.

In addition, the presence of contralateral labeled tectal terminations suggests bilateral tectal control over cMRF neurons. This result is consistent with previous anatomical studies that report a small collicular projection through the tectal commissure to the contralateral MRF (Kawamura and Brodal 1974; Harting et al. 1980; Olivier et al. 1998). The bilateral tecto-reticular pathway may serve to coordinate the cMRF neuronal activity of each side, since the leftward and rightward saccades are coded on separate sides of the brainstem cMRF (Cohen et al. 1986; Waitzman et al. 1996; Handel and Glimcher 1997). In fact, tonic activity in cMRF neurons is generally inhibited during ipsiversive saccades. Perhaps the ipsilateral tecto-reticular projection is excitatory, while the contralateral projection is inhibitory or ends on inhibitory interneurons in the cMRF (although the present data does show direct inputs to reticulotectal cells).

A bilateral projection from the cMRF to the superior colliculus is also demonstrated in the present study, for cMRF neurons on both sides were retrogradely labeled following unilateral superior colliculus injections. This result is consistent with previous findings in both the cat (Grantyn et al. 1982) and monkey (Moschovakis et al. 1988b). In the monkey, it has been shown that axons from cMRF neurons run dorsally and caudally to enter the superior colliculus and then cross in the intertectal commissure to reach the contralateral colliculus (Moschovakis et al. 1988b). While coursing through the colliculi, these axons issue collaterals on both sides that terminate mainly in the intermediate gray layer (SGI). This bilateral tectal projection from the cMRF, shown here to receive a direct input from the superior colliculus, may serve a general role of informing both colliculi about the activity recently generated by the colliculus on one side.

Functions of the cMRF in saccade control

Based on its cell activity and its connections with other oculomotor-related structures, three different hypotheses have been put forward for the role of cMRF neurons in horizontal saccade control. They are the saccade-triggering, the feedback, and the feedforward hypotheses. The possibility of a trigger function is supported by physiological studies that analyzed the discharge pattern of cMRF neurons in relation to the saccade onset (Cohen et al. 1986; Moschovakis et al. 1988b; Kaneko and Langer 1990; Waitzman et al. 1996). The latency between the peak discharge of cMRF neurons and the onset of a saccade has been reported to be either the same or slightly shorter than the latency for the saccade-related burst neurons in the superior colliculus (Moschovakis et al. 1988b; Waitzman et al. 1996). Thus, the timing is appropriate for triggering a saccade. The triggering hypothesis gains additional support from anatomical studies that indicate a direct projection from the cMRF to the nucleus raphe interpositus, which contains omnipause neurons (Edwards 1975; Langer and Kaneko 1984, 1990; Büttner-Ennever and Büttner 1988). The present study suggests that the non-tectoreticular cMRF cells receive tectal input. If the descending cMRF ouput is inhibitory, it could presumably turn off the omnipause gate, allowing presaccadic burst neurons in the pontine gaze center

to fire. Further study is needed to test whether cMRF-raphe neurons do indeed receive collicular input.

The feedforward hypothesis proposes that the cMRF plays a role in the transmission of saccade signals from the superior colliculus to the horizontal gaze centers (Sparks and Mays 1983, 1990; Cohen et al. 1986; Moschovakis et al. 1988b; Moschovakis and Highstein 1994; Waitzman et al. 1996). In this context, the cMRF may provide a type of spatial filter for the collicular output; i.e., cMRF cells may decompose the original oblique saccade vector defined by the superior colliculus and extract the horizontal component (Sparks 1986; Sparks and Mays 1990; Waitzman et al. 1996). The signal specifying this horizontal component would then be fed forward to the pons. The physiological evidence for this proposal lies in the fact that the discharge rates of many cMRF neurons are well correlated with horizontal, but not vertical, saccade amplitudes (Waitzman et al. 1996). However, although tectal projections to the cMRF have been documented in many reports (Cohen and Büttner-Ennever 1984; Moschovakis et al. 1988a, 1988b), and tectal terminations were found in close proximity to non-labeled neurons in the present study, it has not been directly proven that these cells project caudally. Furthermore, tracer studies in both the monkey (Büttner-Ennever and Büttner 1988) and cat (Edwards 1975) did not reveal significant cMRF input to the peri-abducens region, where gaze-related burst neurons are located. Therefore, the evidence that cMRF influences the horizontal gaze changes by a feedforward pathway is incomplete. An alternative circuit that may serve the feedforward hypothesis is provided by projections of the cMRF to the nucleus reticularis tegmenti pontis (Edwards 1975; Edwards and de Olmos 1976). This would allow access to cerebellar circuits that play upon the pontine gaze centers (Gonzalo-Ruiz et al. 1988; Suzuki et al. 1994), as well as feed back to the superior colliculus (May et al. 1990).

The feedback hypothesis proposes that the cMRF relays a current eye displacement signal from pontine premotor centers to the superior colliculus, for use in the dynamic control of gaze changes (Waitzman et al. 1991). It has been reported that there is a nearly linear relationship between the firing rate of a subgroup of cMRF neurons and the decline in dynamic motor error during a saccade (Waitzman et al. 1996). Anatomical studies have shown that the cMRF projects directly to the superior colliculus (Grantyn et al. 1982; Cohen and Büttner-Ennever 1984; Huerta and Harting 1984; Moschovakis et al. 1988b; the present study) and that it receives ascending projections from the paramedian pontine reticular formation (Büttner-Ennever and Henn 1976; Büttner-Ennever and Büttner 1978; Langer and Kaneko 1983). The existence of such a pathway would allow the cMRF to provide the superior colliculus with an efferent copy signal that indicates the current saccade amplitude. The fact that at least a portion of the reticulotectal feedback is inhibitory (Appell and Behan 1990) supports this hypothesis. However, the projection from the pontine reticular formation to the cMRF is an ipsilateral one, while the projections from the cMRF and superior colliculus to the paramedian pontine reticular formation are crossed (Edwards 1975). In view of this anatomical evidence, the source of the activity in the dynamic error subgroup of cMRF neurons is unclear, for the ascending pontine input is from the non-active side of the brainstem. Furthermore, the firing patterns of cMRF reticulotectal cells do not appear appropriate for cells driven by pontine burst neurons (Moschovakis et al. 1988b).

The primary finding of this study is that there is a potent reciprocal connection between the superior colliculus and the cMRF. Furthermore, the signal on tectal, long-lead burst neurons that supply input to the cMRF and cMRF cells projecting to the superior colliculus are virtually indistinguishable in terms of latency and the profile of the burst (Moschovakis et al. 1988b). The most striking difference between identified collicular and reticular cells is that the latter often fire primarily in relation to the horizontal component of the saccade. Thus, the reciprocal connection allows the tectobulbar neurons to provide an efference copy of the saccade command to the cMRF, which then informs *both* colliculi of the activity (primarily related to the horizontal components) that has recently occurred in the colliculus *on one side*. This signal transmission may allow the colliculus to compensate for previous eye movement, a capacity demonstrated by double saccade paradigms (Sparks and Mays 1983; Sparks and Porter 1983). On the other hand, it may influence the saccade-related activity of tectal neurons in an ongoing manner. Specifically, this efference copy signal may provide a dynamic inhibitory feedback loop that extinguishes ,activity in selected collicular neurons, or it could provide an excitatory drive to collicular “build up” neurons (Moschovakis et al. 1988b; Munoz and Wurtz 1995; Waitzman et al. 1991, 1996). Clearly, further experiments, particularly experiments determining the target cells of the cMRF reticulotectal axons, are needed to ascertain the function or functions of this feedback circuit.

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References

Appell PP, Behan M (1990) Sources of subcortical GABAergic projections to the superior colliculus in the cat. J Comp Neurol 302:143–158

Araki M, McGeer PL, McGeer EG (1984) Presumptive γ-aminobutyric acid pathways from the midbrain to the superior colliculus studied by a combined horseradish peroxidase-γ-aminobutyric acid transaminase pharmacohistochemical method. Neuroscience 13:433–439

Bender MB, Shanzer S (1964) Oculomotor pathways defined by electric stimulation and lesions in the brain stem of the monkey. In: Bender MB (ed) The oculomotor system. Harper and Row, New York, pp 81–140

Büttner-Ennever JA, Henn V (1976) An autoradiographic study of the pathways from the pontine reticular formation involved in horizontal eye movements. Brain Res 108:155–164

Büttner-Ennever JA, Büttner U (1978) A cell group associated with vertical eye movements in the rostral mesencephalic reticular formation of the monkey. Brain Res 151:31–47

Büttner-Ennever JA, Büttner U (1988) The reticular formation. In: Büttner-Ennever JA (ed) Neuroanatomy of the oculomotor System. Elsevier, New York, pp 119–176

Cohen B, Büttner-Ennever JA (1984) Projections from the superior colliculus to a region of the central mesencephalic reticular formation (cMRF) associated with horizontal saccadic eye movements. Exp Brain Res 57:167–176

Cohen B, Waitzman DM, Büttner-Ennever JA, Matsuo V (1986) Horizontal saccades and the central mesencephalic reticular formation. Prog Brain Res 64:243–256

Edwards SB (1975) Autoradiographic studies of the projections of the midbrain reticular formation: descending projections of nucleus cuneiformis. J Comp Neurol 161:341–358

Edwards SB, Olmos JS de (1976) Autoradiographic studies of the projections of the midbrain reticular formation: ascending projection of nucleus cuneiformis. J Comp Neurol 165:417–432

Fukushima K, Ohashi T, Fukushima J, Kaneko CRS (1995) Discharge properties of vestibular and saccade neurons in the rostral midbrain of alert cats. J Neurophysiol 73:2129–2143

Gonzalo-Ruiz A, Leichnetz GR, Smith DL (1988) Origin of the cerebellar projections to the region of the oculomotor complex, medial pontine reticular formation and superior colliculus in New World monkeys: a retrograde horseradish peroxidase study. J Comp Neurol 268:508–526

Graham J (1977) An autoradiographic study of the efferent connections of the superior colliculus in the cat. J Comp Neurol 173:629–654

Grantyn A, Grantyn R (1982) Axonal patterns and sites of termination of cat superior colliculus neurons projecting in the tecto-bulbo-spinal tract. Exp Brain Res 46:243–256

Grantyn A, Grantyn R, Berthoz A, Ribas J (1982) Tectal control of vertical eye movements: a search for underlying neuronal circuits in the mesencephalon. In: Roucoux A, Crommelinck M (eds) Physiological and pathological aspects of eye movements. Junk, The Hague, pp 337–344

Grantyn R (1988) Gaze control through superior colliculus: structure and function. In: Büttner-Ennever JA (ed) Neuroanatomy of the oculomotor system. Reviews of oculomotor research, vol II. Elsevier, New York, pp 273–333

Grofova IN, Ottersen OP, Rinvik E (1978) Mesencephalic and diencephalic afferents to the superior colliculus and periaqueductal gray substance demonstrated by retrograde axonal transport of HRP in the cat. Brain Res 146:205–220

Handel A, Glimcher PW (1997) Response properties of saccade-related burst neurons in the central mesencephalic reticular formation. J Neurophysiol 78:2164–2175

Harting JK (1977) Descending pathways from the superior colliculus: an autoradiographic analysis in the Rhesus Monkey (*Macaca mulatta*). J Comp Neurol 173:583–612

Harting JK, Huerta M, Frankfurter A, Strominger L, Royce GJ (1980) Ascending pathways from the monkey superior colliculus: an autoradiographic analysis. J Comp Neurol 192:853–882

Huerta MF, Harting JK (1984) The mammalian superior colliculus: studies of its morphology and connections. In: Vanegas H (ed) Comparative neurology of the optic tectum. Plenum Press, New York, pp 687–773

Kaneko CRS, Fukushima K (1998) Discharge characteristics of vestibular saccade neurons in alert monkeys. J Neurophysiol 79: 835–847

Kaneko CRS, Langer TP (1990) Brainstem afferents to the oculomotor omnipause neurons in monkey. J Comp Neurol 295: 413–427

Kawamura K, Brodal A (1974) The projection of the superior colliculus onto the reticular formation of the brain stem. An experimental anatomical study in the cat. Exp Brain Res 19:1–19

Komatsuzaki A, Alpert J, Harris HE, Cohen B (1972) Effect of mesencephalic reticular formation lesions on optokinetic nystagmus. Exp Neurol 34:522–534

Langer TP, Kaneko CRS (1983) Efferent projections of the cat oculomotor reticular omnipause neuron regions: an autoradiographic study. J Comp Neurol 217:288–306

Langer TP, Kaneko CRS (1984) Brainstem afferents to the omnipause region in the cat: a horseradish peroxidase study. J Comp Neurol 230:444–458

Langer TP, Kaneko CRS (1990) Brainstem afferents to the oculomotor omnipause neurons in monkey. J Comp Neurol 295: 413–427

May PJ, Hartwich-Young R, Nelson J, Sparks DL, Porter JD (1990) Cerebellotectal pathways in the macaque: implications for collicular generation of saccades. Neuroscience 36:305–324

Moschovakis AK, Highstein SM (1994) The anatomy and physiology of primate neurons that control rapid eye movements. Annu Rev Neurosci 17:465–488

Moschovakis AK, Karabelas AB, Highstein SM (1988a) Structure-function relationships on the primate superior colliculus. I. Morphological classification of efferent neurons. J Neurophysiol 60:232–262

Moschovakis AK, Karabelas AB, Highstein SM (1988b) Structure-function relationships in the primate superior colliculus. II. Morphological identity of presaccadic neurons. J Neurophysiol 60:263–298

Nakao S, Shiraishi Y, Li WB, Oikawa T (1990) Mono- and disynaptic excitatory inputs from the superior colliculus to vertical saccade-related neurons in the cat Forel's field H. Exp Brain Res 82:222–226

Munoz DP, Wurtz RH (1995) Saccade-related activity in monkey superior colliculus. I. Characteristics of burst and buildup neurons. J Neurophysiol 73:2313–2333

Olivier E, Porter JD, May PJ (1998) Comparison of the distribution and somatodendritic morphology of tectotectal neurons in the cat and monkey. Vis Neurosci 15:1–20

Scudder CA, Moschovakis AK, Karabelas AB, Highstein SM (1996) Anatomy and physiology of saccadic long-lead burst neurons recorded in the alert squirrel monkey. I. Descending projections from the mesencephalon. J Neurophysiol 76:332–352

Shiraishi Y, Nakao S (1994) Projections of vertical eye movement-related and head rotation-related neurons in the medial mesodiencephalic junction to pontine reticular formation in cat. Neurosci Lett 171:85–88

Sparks DL (1986) Translation of sensory signals into commands for control of saccadic eye movements: role of primate superior colliculus. Physiol Rev 66:118–171

Sparks DL, Mays LE (1983) Spatial localization of saccade targets. I. Compensation for stimulation-induced perturbations in eye position. J Neurophysiol 49:45–63

Sparks DL, Mays LE (1990) Signal transformations required for the generation of saccadic eye movements. Annu Rev Neurosci 13:309–336

Sparks DL, Porter J (1983) Spatial localization of saccade targets. II. Activity of superior colliculus neurons preceding compensatory saccades. J Neurophysiol 49:64–74

Suzuki DA, Yamada T, Betelak K, Yee RD (1994) Ocular motor functions of monkey nucleus reticularis tegmenti pontis. In: Fuchs A, Brandt T, Büttner U, Zee DS (eds) Contemporary oculomotor and vestibular research: a tribute to David A. Robinson. Georg Thieme Verlag, New York, pp 244–246

Szentagothai J (1943) Die zentrale Innervation der Augenbewegungen. Arch Psychiatr Nervenkr 116:721–760

Waitzman DM, Ma TP, Optican LM, Wurtz RH (1991) Superior colliculus neurons that mediate dynamic characteristics of saccades. J Neurophysiol 66:1716–1737

Waitzman DM, Silakov VL, Cohen B (1996) Central mesencephalic reticular formation (cMRF) neurons discharging before and during eye movements. J Neurophysiol 75:1546–1572

Waitzman DM, Silakov VL, DePalma SR (1997) Paralysis of vertical gaze following unilateral inactivation of the mesencephalic reticular formation (MRF). Soc Neurosci Abstr 23:2368