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Anatomical pathways from the optic tectum to the spinal cord subserving orienting movements in the barn owl

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Summary. Electrical stimulation of the optic tectum in many vertebrate species elicits eye, head or body orienting movements in the direction of the receptive field location recorded at the site of stimulation; in the barn owl, tectal stimulation produces short latency saccadic head movements (du Lac and Knudsen 1990). However, the barn owl, like other avians, lacks a direct projection from the tectum to the spinal cord, implying that less direct connections underlie tectally mediated head movements. In order to determine the pathways by which the tectum gains access to spinal cord circuitry, we searched for overlap regions between tectal efferent projections and the locations of cells afferent to the spinal cord. Tectal efferent pathways and terminal fields were revealed by anterograde labeling using horseradish peroxidase (HRP) or tritiated amino acids injected into the optic tectum. Cells afferent to the spinal cord were identified by means of retrograde labeling using HRP, rhodamine, or rhodamine-coupled latex beads injected into the cervical spinal cord. A comparison of results from the anterograde and retrograde labeling experiments demonstrated several areas of overlap. All of the cell groups that both received heavy tectal input and contained a high proportion of cells projecting to the spinal cord were located in the medial half of the midbrain and rhombencephalic tegmentum, and included the red nucleus, the interstitial nucleus of Cajal, the medial reticular formation, the nucleus reticularis pontis gigantocellularis, and the nucleus reticularis pontis oralis. All of these cell groups receive their tectal input from the medial efferent pathway, one of three major output pathways from the tectum. The other two output pathways (the rostral and the caudal) project to regions containing no more than a few scattered cells that are afferent to the spinal cord. Based on these data and on the functions of homologous cell groups in other vertebrates, we hypothesize that the medial efferent pathway and its brainstem target nuclei are primarily responsible for tectally mediated orienting head movements in the barn owl.

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Introduction

The optic tectum issues motor commands that orient an animal's gaze to the source of sensory stimuli. In many animals the orienting movements often involve coordinated actions of the entire body, whereas in others orienting movements are carried out primarily by the head and eyes. In all cases, however, the direction and size (vector) of the orienting movement is encoded by the site of activity in the tectum, and the movement encoded by each site varies systematically across the tectum to form a map of movement vector (eyes: Robinson 1972; Schiller and Stryker 1972; head: Roucoux et al. 1980; du Lac and Knudsen 1990). For such place-coded information to give rise to a coordinated movement of the body, head and/or eyes, the command signal must be transformed into precise temporal patterns of motor unit activity in appropriate motor pools in the brainstem and spinal cord. At least for the control of eye movements, these dramatic spatiotemporal transformations appear to occur in the nuclei of the brainstem reticular formation (for reviews see Raphan and Cohen 1978; Fuchs et al. 1985).

Various reticular nuclei are also thought to contribute to tectally mediated orienting movements of the head or body. Lesion experiments indicate that direct tectospinal projections are not necessary for the expression of body orienting movements (Dean et al. 1986; Masino and Grobstein 1989a, b). In contrast, lesions of brainstem reticular nuclei, which receive input from the tectum and project to the spinal cord, eliminate components of orienting head or body movements in a variety of species (Sprague et al. 1963; Lawrence and Kuypers 1968; Masino and Grobstein 1989b). Moreover, physiological recording from reticular sites in mammals reveals activity

correlated with parameters of head movement (Grantyn and Berthoz 1987), and stimulation of these reticular sites can cause head orienting movements (Hinsey et al. 1930; Ingram et al. 1932; Tehovnik and Yoemans 1986).

In order for tectal activity to elicit head turning behavior, the place-coded motor commands from the optic tectum must be transformed into an appropriate pattern of motoneuron activity in the cervical spinal cord. We chose to study the processes underlying this transformation in the barn owl, a species that relies almost entirely on rapid head movements to redirect gaze. The kinetics of the owl's head movements are similar to those of saccadic eye movements in monkeys and cats; hence, they are referred to as head saccades (du Lac and Knudsen 1990). Focal microstimulation of the optic tectum evokes head saccades at a short latency (20 ms), indicating that fairly direct pathways from the optic tectum to neck motoneurons transform the tectal place code into a coordinated sequence of motor control signals for mediating head movements of appropriate direction, size and kinetics. In this study, we used anatomical techniques to define the most direct pathways from the optic tectum to the cervical spinal cord, pathways that are the most likely candidates for carrying out these transformations.

Materials and methods

Analysis of tectal efferents

Anterograde tracers were injected into the optic tectum in seven owls: the tecta of three owls were injected with horseradish peroxidase (HRP, Sigma type VI), two with tritiated leucine, and one with tritiated proline. For the HRP injections, one eye was enucleated while the owl was anesthetized using halothane 2% and nitrous oxide · 8 l/min. A small hole was drilled in the back of the orbit and the rostral pole of the tectum was exposed. A Gelfoam sponge, saturated with HRP, was placed into the exposed tectal lobe. After two days, the owl was overdosed with sodium pentobarbital. Heparin sulfate was injected into the heart immediately prior to perfusion to reduce clotting, and lidocaine hydrochloride (12.5 ml/l) was added to the clearing solution (0.1 M phosphate buffer; pH 7.4) to dilate the vasculature. After the blood had cleared, the owl was perfused with 1 l of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.05 M phosphate buffer (pH 7.4) with 5% sucrose, followed by an additional 500 ml of the same fixative with 10% sucrose. The brain was blocked, removed from the skull, and soaked in the same fixative solution with 30% sucrose for an additional 4 h. The brain was placed in 30% albumin and 3% gelatin, which was then hardened in 4% formaldehyde for 1 h, and the block was allowed to sink in 30% sucrose phosphate buffer (pH 7.4). The next day, frozen sections were cut at 50 μm, the sections were collected in cold buffer with 30% ethylene glycol and were stored in a freezer. The sections were reacted with tetra methyl benzidine (TMB) according to a modification (Lane 1978) of Mesulum's method (1975) and were mounted on gelatin-coated slides for viewing.

Three owls received multiple (2 or 3) injections of tritiated leucine or proline (50 μCi/μl in saline) into one tectal lobe. Each owl was anesthetized with ketamine hydrochloride (i.m. 15 mg/kg per hour) and valium (i.m. 0.6 mg/kg per hour) and was mounted in a stereotaxic apparatus. A glass micropipette, glued onto a Hamilton syringe filled with label, was positioned in the tectum on the basis of the location of the visual receptive field of units recorded through the pipette. Once in place, 0.2 μl of the tritiated amino acid solution was slowly pressure ejected. The pipette remained in place for 15 min and then was withdrawn. After 5 days, the animals were killed by overdosing with sodium pentobarbital and perfused as above but with formalin in phosphate buffer. Sections were mounted onto subbed slides, dipped in NTB–2 emulsion, and left in the dark for 6 weeks. Afterwards, the slides were developed using Kodak D–19 developer.

Camera lucida drawings of each injected brain were made at 300 μm intervals throughout the labeled sections. The cell groups were defined according to the descriptions by Craigie (1928), Ariens-Kappers et al. (1936), Jungherr (1945), Karten and Hodos (1967) and Cabot et al. (1982).

Analysis of spinal cord afferents

Seven owls received injections of retrograde tracers into the spinal cord: HRP in three cases, rhodamine (Sigma, rhodamine B isothiocyanate) in two cases, and rhodamine-coupled latex beads in two cases. The birds were anesthetized with halothane and nitrous oxide and the vertebral column was exposed dorsally along the midline just caudal to the cranium. The head was held in place using a head bolt, and a small hole was drilled in either the C2 or C3 vertebra to provide access to the spinal cavity. A glass micropipette containing either 20% HRP or H_2O saturated with rhodamine was attached to the end of a 1 μl Hamilton syringe as before. The micropipette was inserted 2–3 mm into the spinal cavity 1 mm lateral to the midline. Approximately 0.5 μl of the tracer was pressure ejected slowly, and the pipette was retracted. In each case, a second injection was made just caudal to the first. Brains containing rhodamine were fixed and sectioned as described for autoradiography, and coverslipped with a non-fluorescing medium (Krystalon). Cells filled retrogradely with HRP or rhodamine were plotted onto transverse atlas sections of the brainstem.

Results

Afferent projections to the spinal cord

The patterns of retrograde labeling from HRP or rhodamine injections into the cervical spinal cord (C3–C4) were qualitatively similar, although rhodamine tended to label more cell bodies than did HRP or rhodamine-coupled beads. In every case, the injection was large, encompassing most or all of one side of the spinal cord. Cases ($n=2$) in which the label spread to the opposite side were not included in the study. While there was significant variation in the number of cells labeled in different nuclei across the different cases, those nuclei that did contain label did not vary.

Telencephalon. Retrogradely filled cells were observed in the contralateral medial hyperstriatum following spinal cord injections of HRP. Most were medial to the lateral ventricle in a region that extended from the caudalmost aspect of the hyperstriatum, without including the hippocampus, to a level about two-thirds of the way to the frontal poles.

While there was no indication that the injection sites invaded the medulla, the possibility that there was some uptake from medullary sites could not be entirely ruled out given the difficulty in distinguishing between high cervical spinal cord and caudal medulla. (This issue is important when interpreting the telencephalic labeling

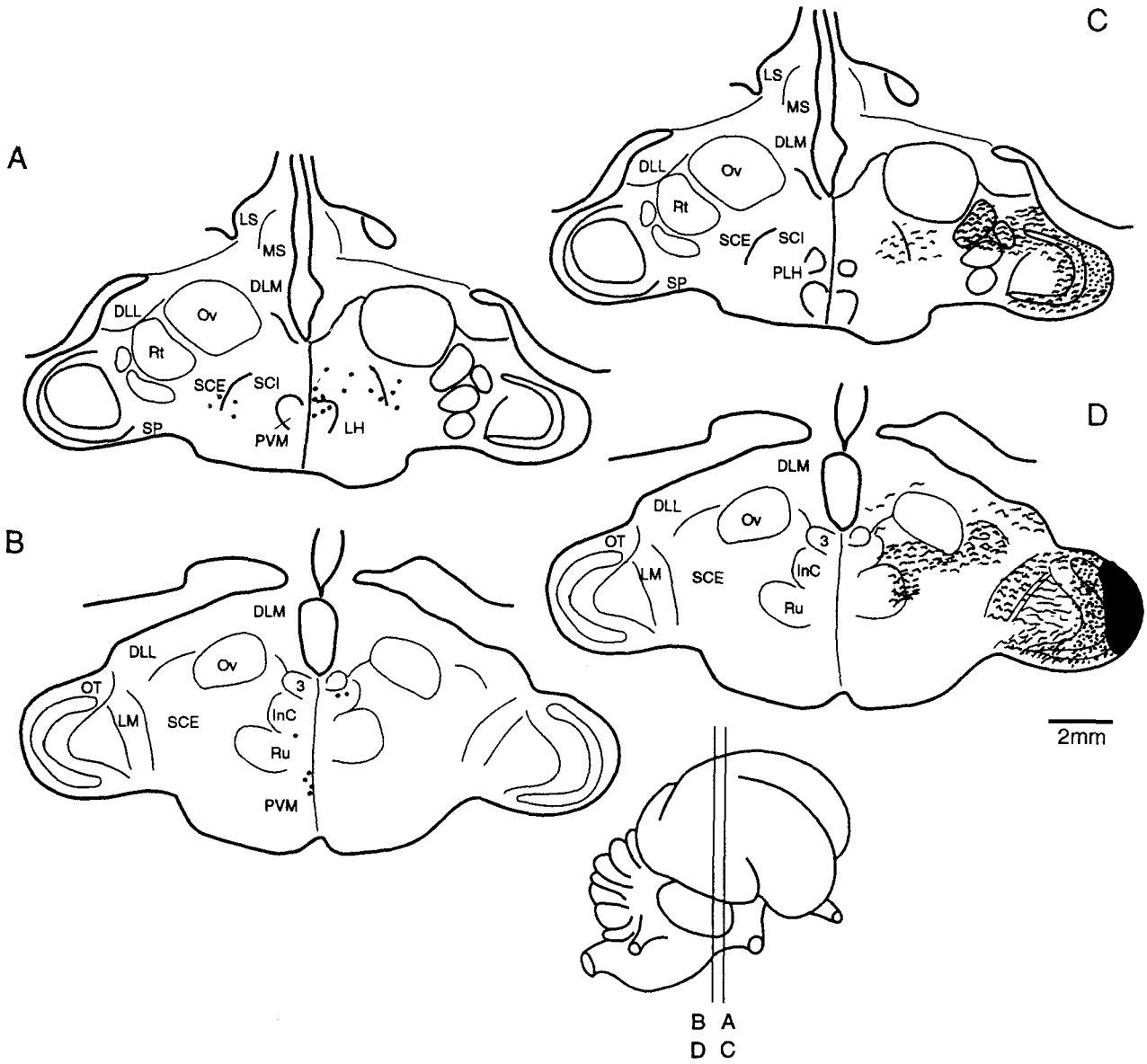

Fig. 1A–D. Camera lucida drawings of labeling following HRP injection into the cervical spinal cord (**A, B**) or right lobe of the optic tectum (**C, D**). The rostrocaudal levels of the transverse sections are shown at the *bottom right*, with **A** and **C** rostral and **B** and **D** caudal. Note that **A** and **C** are identical levels of section, as are **B** and **D**. Spinal afferent labeling is restricted to regions in and adjacent to the hypothalamus, tectal efferent labeling to the nucleus rotundus and pretectal nuclei

Abbreviations (Figs. 1–9): *3*, oculomotor nucleus; *4*, trochlear nucleus; *5m*, trigeminal motor nucleus; *5s*, trigeminal sensory nucleus; *6*, abducens nucleus; *8*, vestibular nuclei; *Cb*, cerebellum; *CG*, central gray; *Dk*, nucleus Darkschewitsch; *DLL*, lateral dorsolateral thalamic nucleus; *DLM*, medial dorsolateral thalamic nucleus; *DLP*, posterior dorsolateral thalamic nucleus; *DMP*, posterior dorsomedial thalamic nucleus; *DT*, dorsal tegmental nucleus; *EW*, Edinger-Westphal nucleus; *HSm*, medial Hyperstriatum; *IC*, inferior colliculus; *ICo*, *IcO*, intercollicularis nucleus; *InC*, interstitial nucleus of Cajal; *IO*, isthmo-optic nucleus; *IS*, nucleus isthmi, magnocellularis; *IP*, interpeduncular nucleus; *Lam*, nucleus laminaris; *LH*, lateral hypothalamus; *LLD*, nucleus of lateral lemniscus, dorsal; *LLV*, nucleus of lateral lemniscus, ventral; *LM*, nucleus lentiformis mesencephalicus; *LRF*, lateral reticular formation; *LS*, lateral septal nucleus; *MLF*, medial longitudinal fasciculus; *MRF*, medial reticular formation; *MS*, medial septal nucleus; *OT*, optic tectum; *Ov*, nucleus ovoidalis; *PC*, posterior commissure; *PLH*, posterior lateral hypothalamus; *PT*, pretectal nucleus; *PVM*, periventricular hypothalamic nucleus; *RPc*, pontine reticular nucleus, caudal; *RPgc*, pontine reticular nucleus, gigantocellularis; *RPo*, pontine reticular nucleus, oralis; *Rt*, nucleus rotundus; *Ru*, red nucleus; *SCE*, stratum cellular externum; *SCI*, stratum cellular internum; *SLu*, nucleus semilunaris; *SO*, superior olivary nucleus; *SP*, subpretectal nucleus; *SpL*, lateral spiriform nucleus; *TP*, tegmental pedunculo-pontine nucleus; *TTD*, nucleus/tract of trigeminal nerve; *VeM*, medial vestibular nucleus; *VLV*, ventral nucleus of lateral lemniscus

since this projection has not been reported in other birds [Webster and Steeves 1988]).

Diencephalon. Three regions of the diencephalon were labeled retrogradely by spinal cord injections (Fig. 1). Most prominent was the ipsilateral magnocellular periventricular nucleus (PVM) in the caudal hypothalamus. This well-circumscribed, large-celled nucleus contained many labeled cells in each case. Additionally, scattered cells were seen in the lateral hypothalamus

(LH), an area lying immediately lateral and rostral to the PVM. Some cells were labeled in the stratum cellulare externum (SCE), a ventral thalamic nucleus. These cells were morphologically similar to and continuous with the lateral hypothalamic cells and, as such, may represent the same population.

Mesencephalon. Small numbers of retrogradely labeled cells were present in the contralateral tectal lobe in three of the seven cases; the most cells counted in any single case was nine. In each case of labeled tectal cells (e.g. Fig. 2F), the cells were located in deep tectal layers (layers 11–15, Ramon y Cajal 1972).

In contrast to the sparse labeling in the tectum, many labeled cells were observed in nuclei of the midbrain tegmentum (Fig. 2). Most of the cells were situated near the midline around the medial longitudinal fasciculus (MLF). In this region, it was almost exclusively the large cells that were labeled. Moreover, in some injection cases, each of the large cells was labeled, suggesting that these, and not the more numerous small cells, form the projection to the spinal cord.

Most of the labeled medial tegmental cells were within one of four midline tegmental cell groups: the interstitial nucleus of Cajal (InC), the medial reticular formation, the nucleus Darkschewitsch (DK), and the red nucleus (Ru). Labeled cells within the Dk, a small cluster of medium and large cells within the midbrain central gray (CG) just dorsal to the InC (Fig. 3A), were observed throughout its ipsilateral extent (Fig. 2A–B). An example of the labeling pattern within the ipsilateral Dk is shown in Figure 3B.

The InC was defined as the cluster of medium and large cells in the medial midbrain tegmentum, ventral to the CG and lateral to the midline oculomotor complex (Fig. 3A). This definition encompassed a broad region of the medial tegmentum, and it is likely that this region contains several cell groups that, in other species, have been given other names, such as the nucleus of the medial longitudinal fasciculus, the ventral tegmental nucleus, and the pre-rubral fields (Ariens Kappers et al. 1936; Graybiel 1982; Cabot et al. 1982; Holstege and Crowie 1989). Each of these nuclei shares with the InC the ipsilateral descending projection to the spinal cord. However, in the barn owl we found no anatomical basis for subdividing this region. Most of the large cells and many of the medium sized cells within the InC projected ipsilaterally to the spinal cord (Fig. 2A–D). Examples of labeled large and medium InC cells are shown in Fig. 3C. The large cells typically contained large branching dendritic processes which extended throughout the InC as well as into the laterally adjacent medial reticular formation (MRF).

The MRF is a region lateral and caudal to the InC that contains sparsely packed, medium sized cells (Fig. 3A). In each case, several labeled cells were found in the ipsilateral MRF (Fig. 2A–E), while fewer cells were found in the contralateral MRF (Fig. 2B–D). Examples of the labeled MRF cells are shown in Fig. 3D. These cells were similar in morphology to neighboring InC cells, although the MRF cells tended to be smaller.

The Ru was defined as the region ventral to the InC in the medial third of the midbrain tegmentum that contains cells which appear dark with Nissl staining (Fig. 4A). The Ru extended approximately 2 mm laterally from the midline. The cells were not tightly clustered, as they are in pigeons (Karten and Hodos 1967). Following spinal cord HRP injection, labeled cells in the contralateral Ru appeared throughout its rostro-caudal extent, but tended to be more numerous in the medial aspects of the nucleus (Fig. 2B–F). While most of the Ru labeling was contralateral, some labeled ipsilateral cells were observed (Fig. 2B, E). Examples of labeled cells in the Ru are shown in Fig. 4B.

In two cases of spinal cord rhodamine injection, but not in cases of HRP injection, cells in the oculomotor complex were labeled, implying the existence of axonal collateral to spinal levels.

Rhombencephalon. Several reticular nuclei in the rostral rhombencephalon were labeled retrogradely by spinal cord injections. The nucleus reticularis pontis oralis (RPo), the rostralmost rhombencephalic portion of the reticular formation (Fig. 5A), contained labeled cells bilaterally in most cases (eg., Fig. 5F, G; no RPo label was seen in the case illustrated in Fig. 6). The labeled cells were observed scattered throughout the RPo region, and tended to be fusiform or unipolar (Fig. 5B).

The nucleus reticularis pontis gigantocellularis (RPgc), a diffuse group of large and medium sized cells in the ventromedial rhombencephalon caudal to the RPo (Fig. 5C), consistently contained many labeled cells (Fig. 5G–H). The labeling in this cell group was also bilateral, with a tendency for the ipsilaterally labeled cells to be located closer to the midline than the contralaterally labeled cells. Labeled RPgc cells tended to be large and multipolar (Fig. 5D).

Further caudal, most regions of the brainstem reticular formation contained cells projecting to the spinal cord, including the nucleus pontis caudalis (Fig. 6F). In addition to the general reticular formation labeling, labeled cells were seen ipsilaterally in the locus coeruleus and the medial vestibular nucleus, and substantial labeling occurred in the internal cerebellar nucleus, the nucleus of the descending trigeminal tract. Bilateral labeling was observed in the lateral vestibular nucleus, the peri-MLF and peri-trochlear regions, and the midline raphe complex.

Efferent projections of the optic tectum

HRP and tritium labeling revealed different aspects of the tectal efferent projections. The HRP material was better for resolving details of the morphology of the processes which enabled terminal fields to be distinguished from fibers of passage. Terminal fields were identified on the basis of labeled bouton-like swellings and filled axons that branched or enveloped cell bodies or proximal dendrites. The tritium material demonstrated best the boundaries of the tracts and terminal fields.

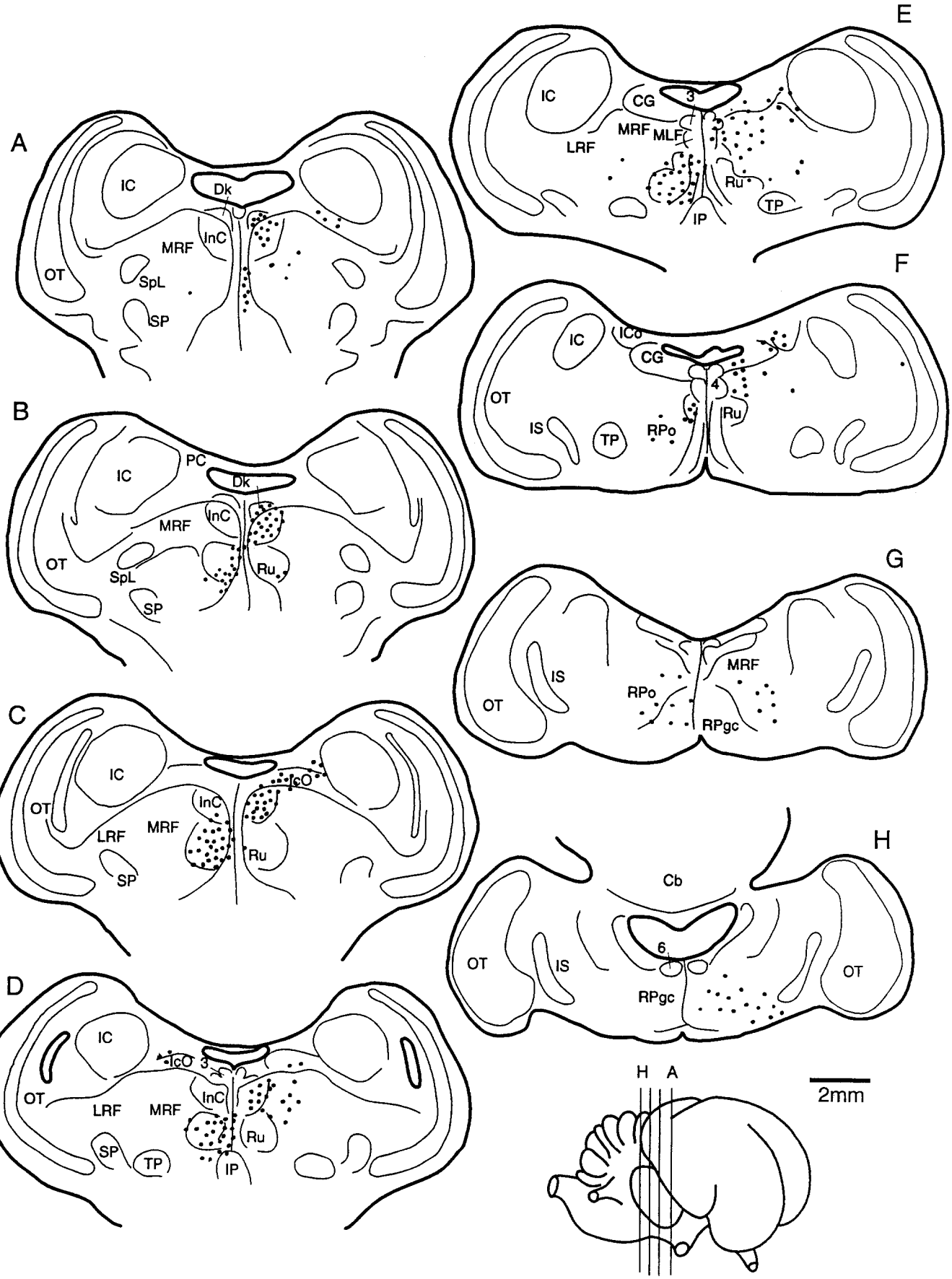

Fig. 2A–H. Locations of retrogradely filled cell bodies from one case of HRP injection into the right cervical spinal cord drawn on transverse sections of the brain. Rostral mesencephalic level (**A**) through rostral rhombencephalic level (**H**). Each dot represents a labeled cell body. These sections are similar to those on which the tectal efferent label was plotted (Fig. 7) to facilitate comparison. The plane of section, shown by *inset at bottom right*, represents the transverse plane through the mesencephalon. The transverse plane is different from that used for the thalamus (Fig. 1) and rhombencephalon (Figs. 6, 8) due to the curvature of the brain through mesencephalic regions

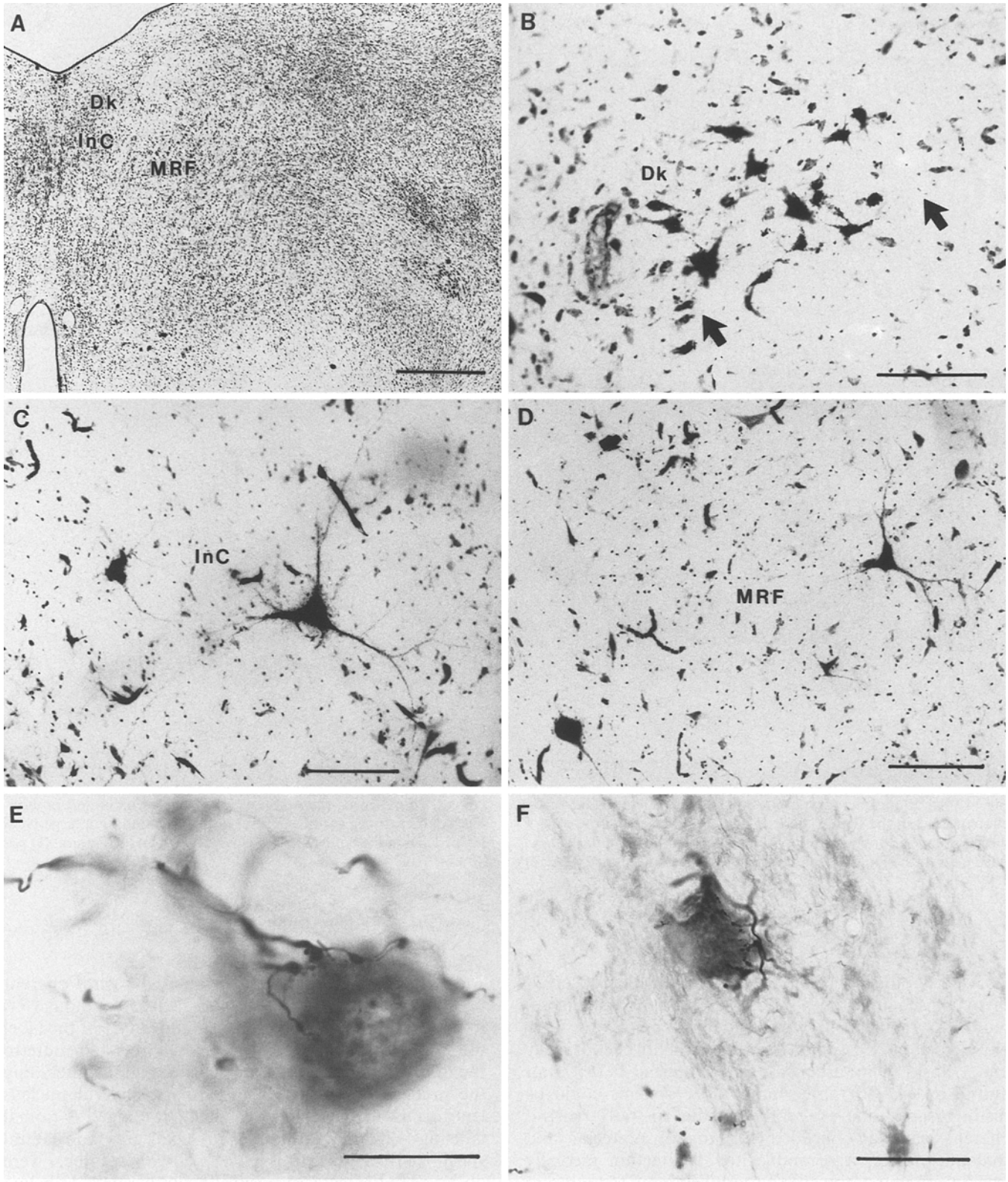

Fig. 3A–F. Photomicrographs of spinal cord afferent and tectal efferent label in the rostral mesencephalon. **A** Nissl stained section through the rostral mesencephalon showing the relative locations of InC, Dk and MRF. Dk is within the CG region, while InC is ventral to it and lateral to the midline oculomotor cells. More lateral, among bands of dense fibers, is situated the MRF. *Bar* 1 mm. **B** Backfilled cells in the Dk following HRP injection into the ipsilateral cervical spinal cord. *Arrows* represent the medio-lateral extent of Dk. *Bar* 150 μm. **C** Example of labeled cells in the ipsilateral InC from spinal cord HRP injection. *Bar* 100 μm. **D** Labeled cells in the MRF from spinal cord HRP injection. *Bar* 100 μm. **E** HRP labeled axon and bouton-like swellings from tectal HRP injection surrounding a large Nissl stained cell in the InC. The InC cell is ipsilateral to the injected tectal lobe. *Bar* 40 μm. **F** Similarly stained axon and cell in the ipsilateral MRF. *Bar* 40 μm

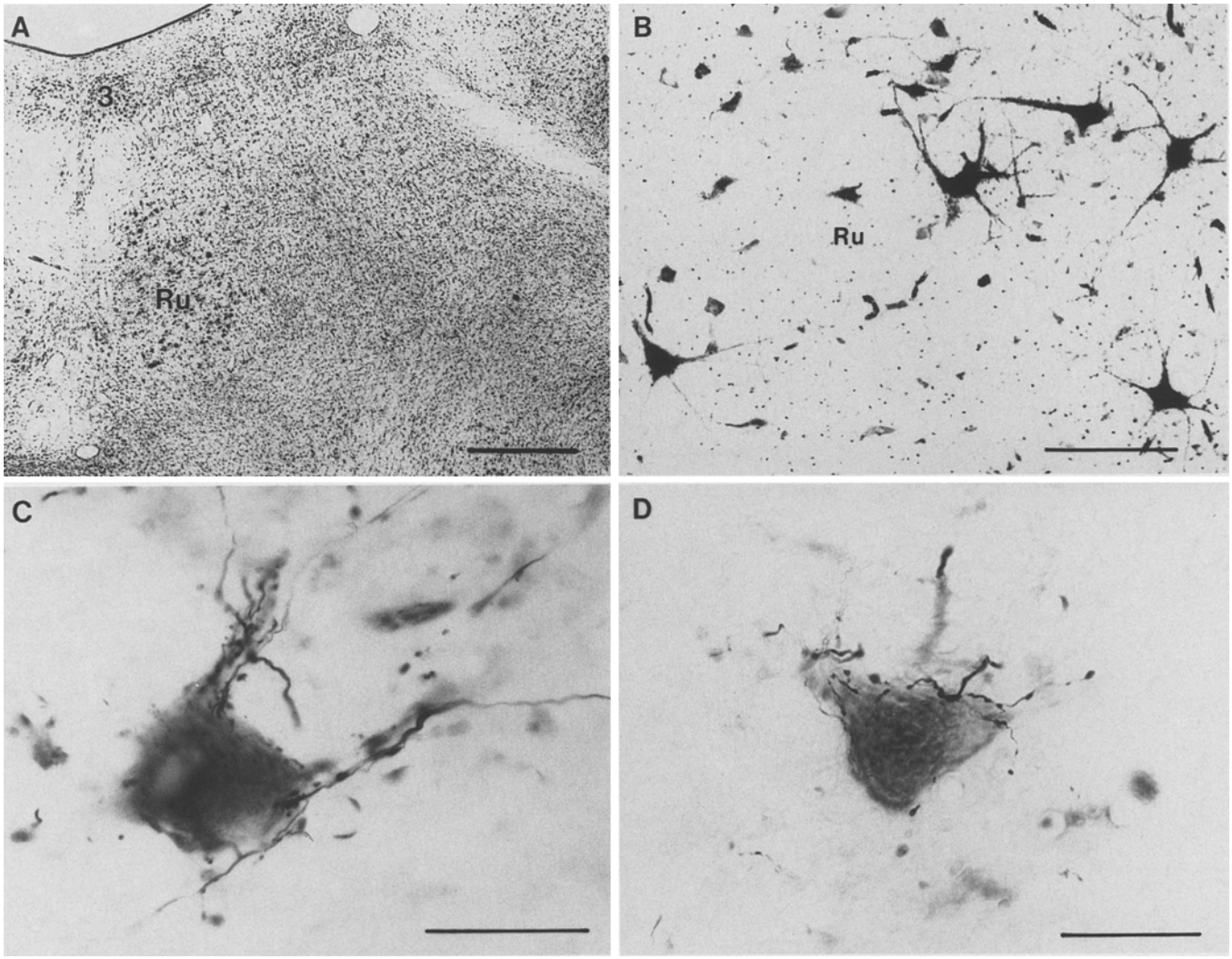

Fig. 4A–D. Photomicrographs of spinal cord afferent and tectal efferent label in the Ru. **A** Nissl stained section through the rostral mesencephalon showing the location of the Ru. *Bar* 1 mm. **B** Backfilled cells in the Ru following HRP injection into the contralateral cervical spinal cord. *Bar* 100 μm. HRP labeled axon and bouton-like swellings from tectal HRP injection surrounding a large Nissl stained cell in the contralateral (**C** *bar* 50 μm) and ipsilateral (**D** *bar* 40 μm) Ru

Anterograde label from the optic tectum was observed in many thalamic, midbrain and rhombencephalic regions. As reported in other vertebrate classes (Ariens-Kappers et al. 1936; Butler and Northcutt 1980; Dacey and Ulinski 1986; Ebbesson and Vanegas 1976; Hunt and Kunzle 1976), three major fiber systems could be distinguished: one exited the tectum rostrally (rostral efferent pathway), headed for rostral pretectal and thalamic nuclei; a second exited the tectum medially (medial efferent pathway) and supplied input to midbrain cell groups on the ipsilateral side before continuing across the midline in the ansular commissure and turning caudally to innervate medial midbrain and rhombencephalic reticular regions contralaterally; and a third exited the tectum caudally (caudal efferent pathway), descending ipsilaterally to the lateral midbrain and rostral pons.

Rostral efferent pathway. Fibers in the rostral efferent pathway projected mainly to the nucleus rotundus (Rt) in the dorsal thalamus (Fig. 1C). While most of the label in the pretectal region appeared to be fibers en route to the dorsal thalamus, several pretectal regions, including the pretectal nucleus (PT) and the ipsilateral nucleus lentiformis mesencephalicus (LM) contained terminal labeling (Fig. 1D), ipsilateral pretectal gray and lateral spiriform nucleus (SpL, Fig. 7A). No labeled fibers were observed in the tectal or posterior commissures. Labeling within the subpretectal nucleus (SP) and regions immediately dorsal appeared in the HRP tectal injections (Fig. 7A–E), but not in the autoradiography cases, implying that such label represents tectal afferent projections or their collaterals.

In two autoradiography cases, a group of fibers exited the tectum ventrally and continued ventrally past the Rt,

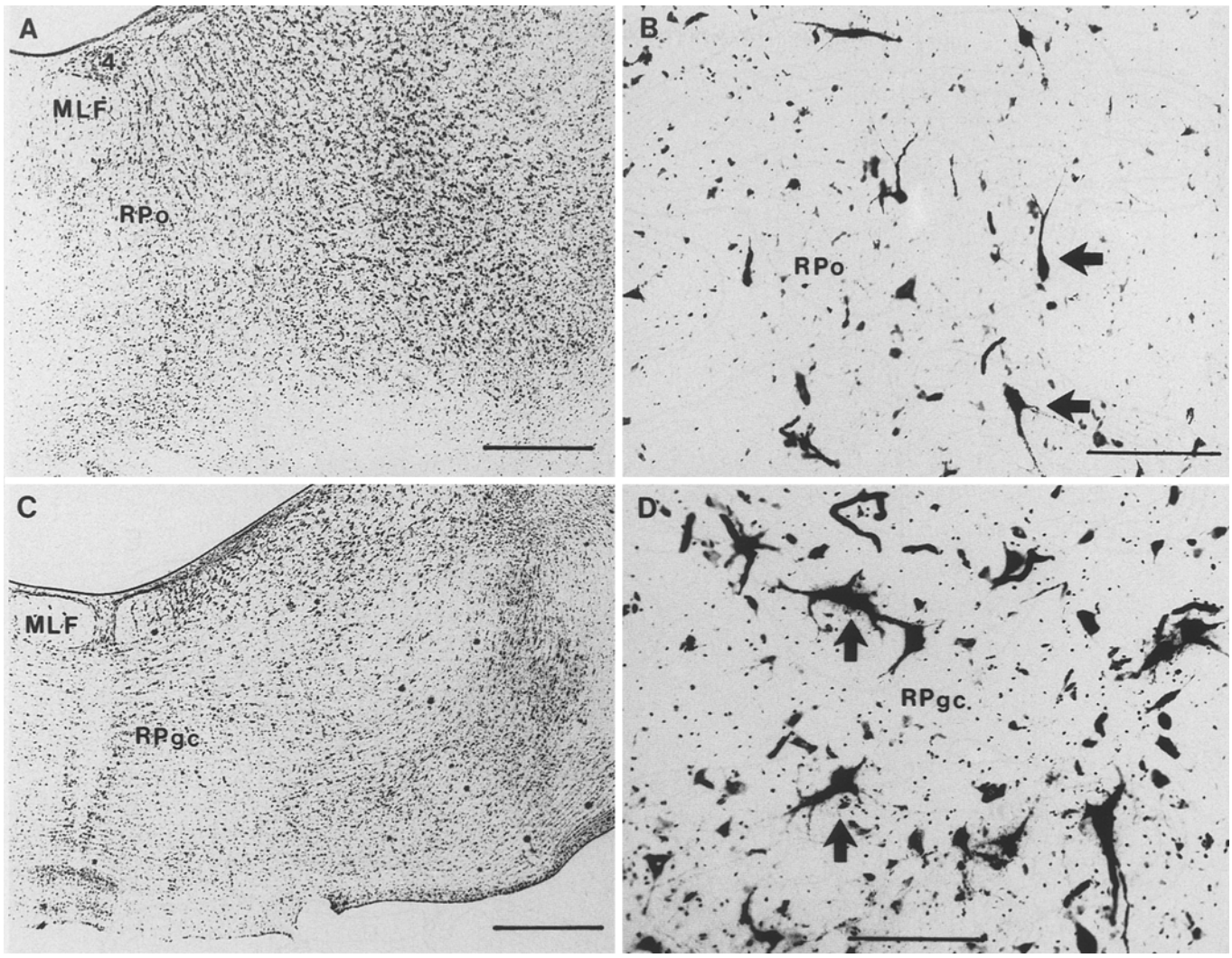

Fig. 5A–D. Photomicrographs of retrograde label in the rhombencephalic reticular formation following HRP injection into the ipsilateral cervical spinal cord. **A** Nissl stained section through the rostral rhombencephalon, showing the location of the nucleus reticularis pontis oralis (*RPo*). *Bar* 1 mm. **B** Retrogradely labeled cells *(arrows)* in the RPo. *Bar* 100 μm. **C** Nissl stained section from a more caudal level in the rhombencephalon, showing the location of the nucleus reticularis pontis gigantocelluaris *(RPgc)*. *Bar* 1 mm. **D** Retrogradely labeled cells in the RPgc. *Bar* 100 μm

crossed the midline in the supraoptic chiasm, and ascended to the contralateral SpL and the rostral portion of the contralateral Rt.

Medial efferent pathway. The medial efferent pathway projected diffusely to a large portion of the midbrain and rhombencephalic reticular formation (Fig. 7). (We chose the term "medial efferent pathway", rather than the more conventional "crossed tectospinal tract", since this pathway has extensive connections with both ipsilateral and contralateral tegmental cell groups, and has, at best, meager connections with the spinal cord.) The medial pathway exited from the central two-thirds of the tectum and coursed ventrally and medially beneath the inferior colliculus (IC). Fibers branched profusely in the lateral and medial ipsilateral midbrain tegmentum as they turned towards nuclei in the medial tegmentum. Fiber branches and bouton-like swellings occurred in and around cells of the Ru, InC, and MRF. (The MRF also contains the dendritic fields of these more medial cell groups; Ariens-Kappers et al. 1936.) Most of the branches stayed within this region, but some were observed to extend into the adjoining Dk, CG and oculomotor complex (oculomotor nucleus and trochlear nucleus) (Fig. 7A–E).

In two cases of large HRP injections into the tectum, many small fibers were seen crossing the midline dorsally through the oculomotor complex into the area of the contralateral InC, Dk, and MRF, where they appeared to terminate. No such dorsally crossing fibers could be traced to the contralateral Ru.

The main fiber bundle crossed the midline ventral to the rostralmost portion of the MLF and also sent fiber branches into the contralateral InC, Ru, and Dk. After crossing, the bundle turned caudad and continued along the medial and ventral aspects of the contralateral

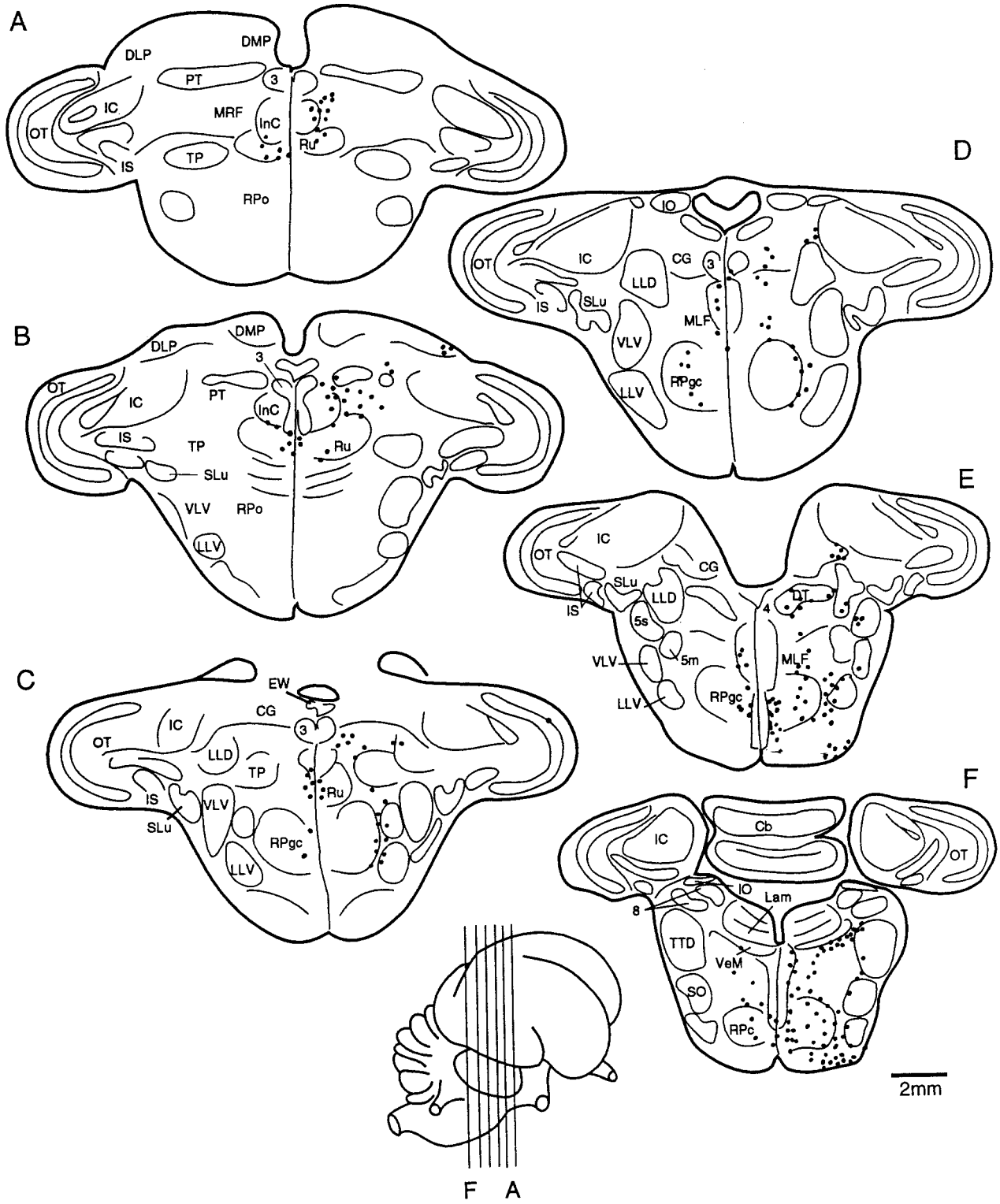

Fig. 6A–H. Locations of retrogradely filled cell bodies in one case of HRP injection into the right cervical spinal cord drawn on transverse sections of the brain. Rostral rhombencephalic level (**A**) through caudal rhombencephalic level (**H**). Each *dot* represents a labeled cell body. The levels of section are shown by the *inset at the bottom right*. The plane of section is the same as that for the thalamus (Fig. 1)

rhombencephalic tegmentum (Fig. 7E–H). Along its descent, fibers emerged from the bundle and penetrated the RPo and RPgc.

Frequently observed in the HRP material were numerous fiber branchings and terminal profiles which enveloped the somata and proximal dendrites of cells in the ipsilateral InC and MRF (Fig. 3E, F) and in the Ru bilaterally (Fig. 4C, D). These profiles suggest that the tectum exerts a powerful influence on the activity of these neurons. In one case of tectal HRP injection, the somas of most of the larger cells in the ipsilateral InC and MRF were surronded by tectal efferent axons. Tracing some of

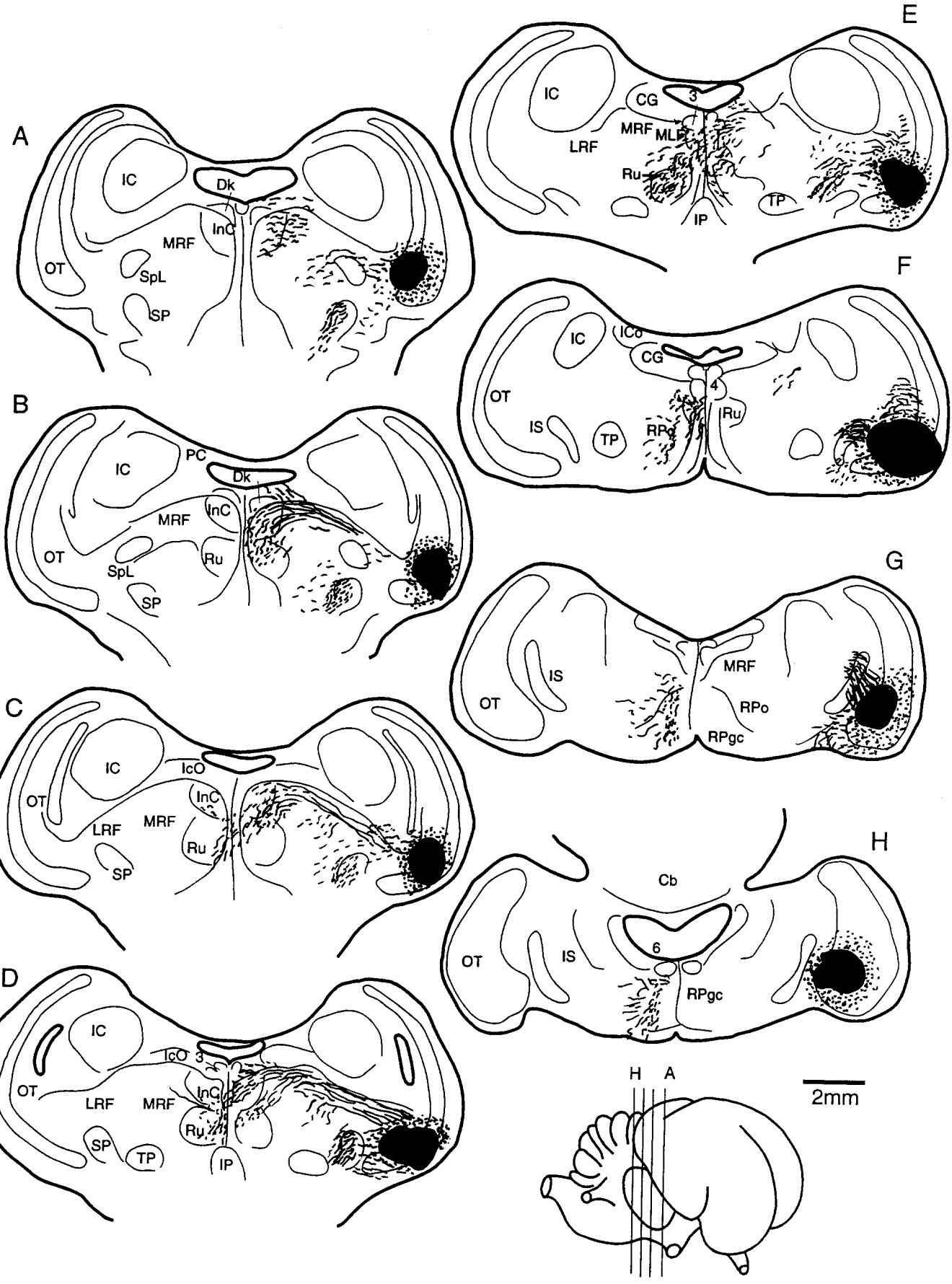

Fig. 7A–H. Locations of tectal efferent fibers in the mesencephalon following HRP injection into the right tectal lobe. For comparison with spinal cord afferent labeling, labeled fibers were drawn onto transverse sections identical to those of the illustrated case of spinal cord HRP injections (Fig. 2). *Inset* rostrocaudal level of the illustrated sections, with **A** the furthest rostral and **H** the most caudal. *Solid black regions* in the right tectum represent site of HRP injection, while surrounding *stipple* represents the region of high background HRP labeling

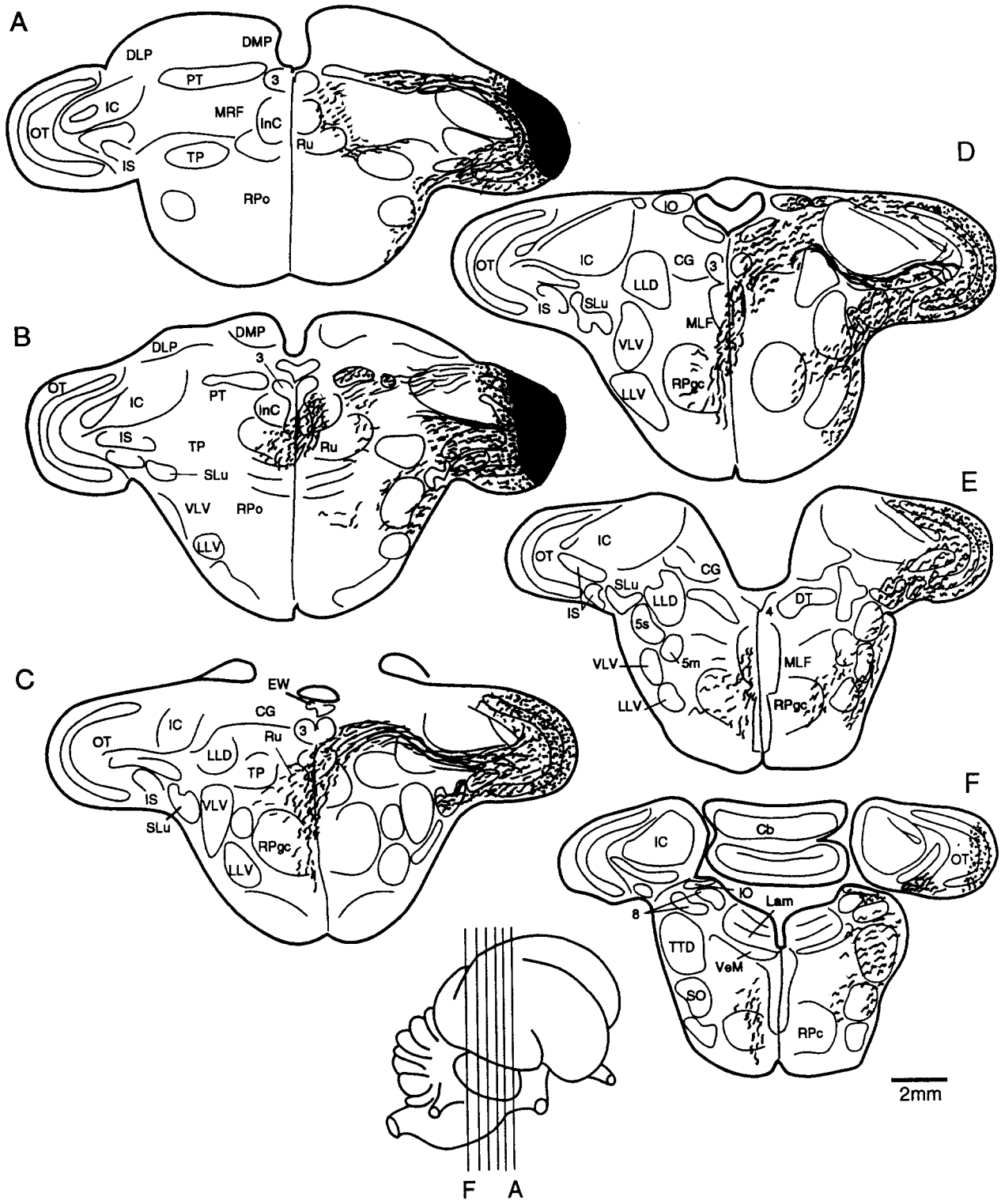

Fig. 8A–F. Locations of tectal efferent fibers in the rhombencephalon from one case of HRP injection into the right tectal lobe. Conventions as in Fig. 7, with transverse sections identical to those in Fig. 6

the individual labeled axons in the ipsilateral InC revealed that single tectal efferent fibers make close contacts with multiple InC cells and that individual InC cells can be surrounded by fibers from more than one tectal efferent branch.

Although there was a gradual decrease in the total amount of labeling along the caudal descent of the medial efferent pathway, there was no indication of more or less branching corresponding to a differential projection to any particular tegmental cell group. In all cases, many fibers persisted to the level of the rostral hypoglossal complex in the caudal rhombencephalon, but none extended as far as the spinal cord.

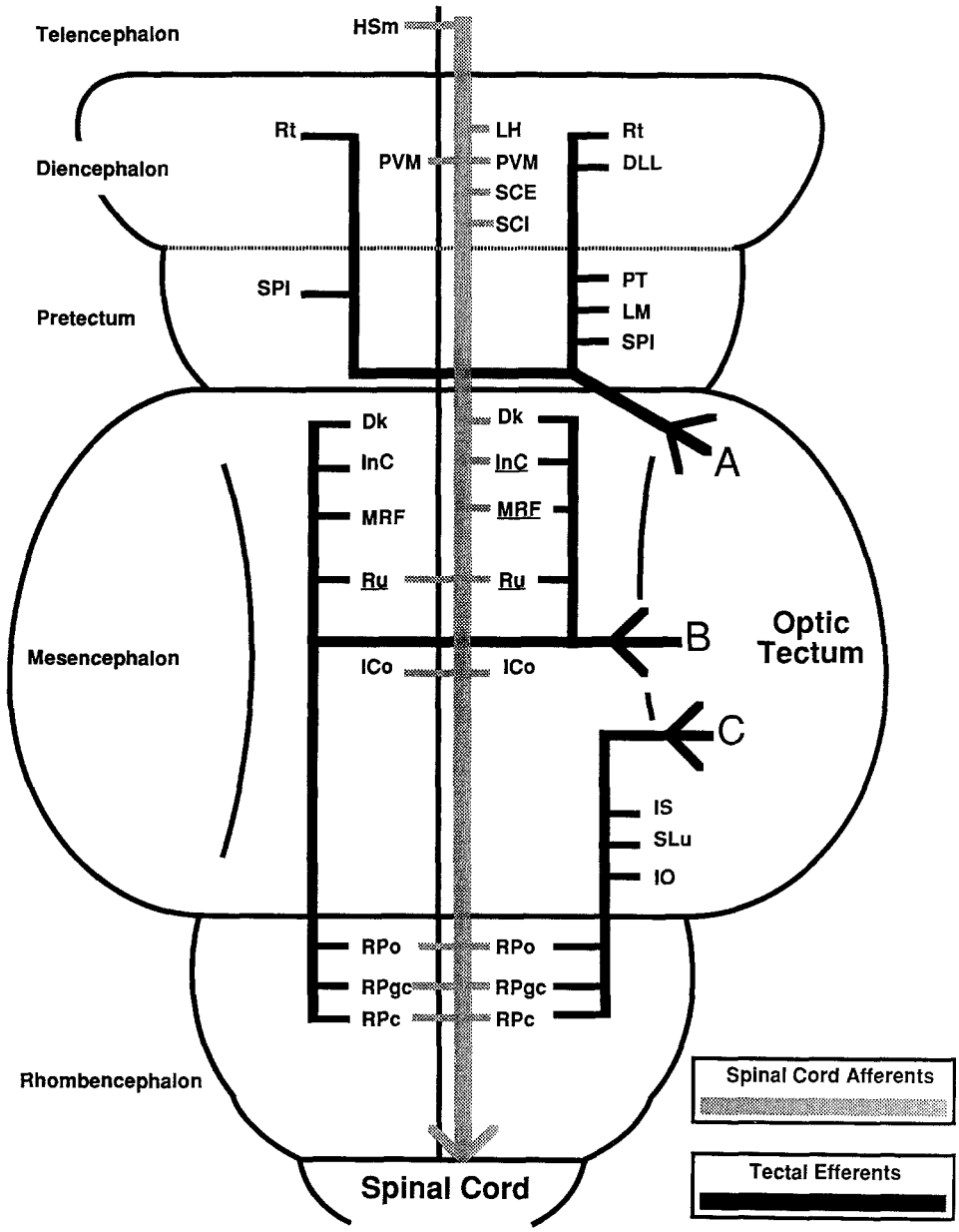

Fig. 9. Summary diagram of tectal efferent and spinal cord afferent projections, highlighting areas of overlap. The regions indicated contained substantive and consistent labelling following injection of HRP or tritiated amino acid into the optic tectum, or cells retrogradely filled following injection of HRP or rhodamine into the spinal cord. *Solid lines* represent tectal efferent connections, *stippled lines* represent spinal cord afferent projections. *Underlined names* indicate those cell groups with evidence of close contacts with tectal efferent fibers (Figs. 3E, F and 4C, D). Tectal efferents divided into three pathways: **A** rostral efferent pathway, **B** medial efferent pathway, and **C** caudal efferent pathway

Caudal efferent pathway. A third major efferent pathway, consisting primarily of small caliber fibers, exited the tectum caudally and ventrally and coursed along the lateral tegmentum just lateral to the trigeminal nuclear complex (Fig. 8). The projection shifted slightly medially along its descent through the mesencephalon, but remained in the lateral reticular formation. These fibers had fewer branches than those of the medial efferent pathway, but exhibited many terminal profiles along their trajectories, suggesting connections with cells in the sub-coeruleus complex (SCv/SCd) and the rhombencephalic reticular nuclei, including RPo, RPgc, and the caudal pontine reticular nucleus (RPc). Cells in both the fifth and seventh cranial nerve nuclei were located near this projection, but there was no evidence of terminal labeling in these nuclei. Further caudally, a dense projection was seen in the ipsilateral isthmo-optic nucleus (IO) just dorsal to the rostral vestibular nucleus (Fig. 8F).

Immediately upon exiting the tectum, fibers traveling with the caudal efferent pathway also entered the ipsilateral nucleus isthmi (IS) (Fig. 7F, G). The labeling consisted of a dense fiber patch restricted to a limited portion of the IS. This is consistent with the topographic tectal projection to this structure observed in other species (Graybiel 1978; Grobstein and Comer 1983). Similar dense fiber patches as well as clusters of retrogradely filled cells appeared in the nucleus semilunaris (SLu) bilaterally (Fig. 8D, E). These patterns of connections between the optic tectum and the IS and SLu are the same as those between the optic tectum and the IS in frogs (Grobstein and Comer 1983), implying that together the IS and SLu in the owl might be homologous to the IS in other classes of vertebrates.

Regions of overlap of tectal efferents with spinal afferents

Tectal efferent projections and spinal cord afferents exhibited several regions of anatomical overlap. Figure 9 is a schematic representation of the regions of direct overlap between tectal efferents and spinal afferents. Five cell groups in the brainstem tegmentum contained both heavy tectal efferent labeling and large numbers of cells projecting to the spinal cord. These were the Ru, InC and MRF in the midbrain and the RPgc and RPo in the rhombencephalon. In the case of the InC and rostral Ru, the boundaries of the tectal efferent labeling corresponded well with the boundaries of the nuclei themselves. Several other regions of overlap contained either sparse terminal labeling from the tectum and/or few cells that labeled from the spinal cord. These were the Dk, mesencephalic CG, and the oculomotor complex in the midbrain and the RPc in the rhombencephalon.

Discussion

This study identifies nuclei that are likely to be involved in tectally mediated head movements. The data demonstrate that a direct projection from the optic tectum to the caudal medulla and cervical spinal cord, which contain motoneurons controlling head movements, is, at best, very slight. This indicates that tectally mediated orienting movements in the barn owl are not controlled by direct tectospinal connections. However, superimposing the regions of tectal efferent terminations (as evidenced by axonal branching, bouton-like swellings, and axonal envelopment of cell bodies and primary dendrites) on the location of spinal afferent cell bodies, reveals that the medial efferent pathway and its connections with regions in the medial brainstem tegmentum establish a short, indirect tecto-tegmento-spinal pathway. This finding, combined with our understanding from other studies of the involvement of these midline cell groups in motor control (Büttner et al. 1977; Raphan and Cohen 1978; Grantyn et al. 1982; Huerta and Harting 1982; Grobstein 1988, 1989, 1990; Masino and Grobstein 1989a; Masino and Knudsen 1992), suggests that the indirect projections to the spinal cord via the medial efferent pathway are primarily responsible for tectally mediated head movements.

Comparison with other avians

The pattern of projections from the optic tectum to the brainstem tegmentum and from the brainstem tegmentum to the spinal cord are similar to those that have been reported in other avian species (Benowitz and Karten 1976; Hunt and Kunzle 1976; Cabot et al. 1982; Webster and Steeves 1988). In these previous studies, the tectal projection to the medial brainstem tegmentum was described, but the InC, Ru, Dk, RPgc, and RPo were not specifically defined. Instead, these nuclei were referred to collectively as the medial reticular formation. As in these other avian species there is, at most, an extremely sparse direct connection between the optic tectum and spinal cord in the barn owl.

There are two notable differences between the tectofugal projections in barn owls and pigeons. In the owl, as opposed to the pigeon, large diameter axons contribute to the medial efferent pathway. These large axons may be a specialization for mediating the owl's highly developed, saccadic head movements, since the axons project to a region from which head movements can be elicited upon electrical microstimulation (Masino and Knudsen 1992). A second difference is the direct intertectal pathway which exists in the pigeon (Benowitz and Karten 1976) but is absent in the owl.

In pigeons, the rostral, medial and caudal efferent pathways originate from different cell types in the optic tectum (Benowitz and Karten 1976; Hunt and Kunzle 1976; Reiner and Karten 1982). Moreover, our data indicate that the cell groups that receive input from the medial pathway project to the spinal cord, whereas the cell groups that receive input from the rostral and caudal pathways, in general, do not. This is consistent with the hypothesis that the medial pathway subserves a function distinct from that of the other two pathways (Reiner and Karten 1982).

Comparison with other vertebrates

The tectofugal pathways in the owl share the general characteristics of the tectofugal pathways in other vertebrate classes (fish: Ebbesen and Vanegas et al. 1976; Butler and Northcutt 1980; amphibians: Masino and Grobstein 1989c; reptiles: Dacy and Ulinski 1986; mammals: Harting 1977; Holcombe and Hall 1981; Grantyn and Grantyn 1982; Grantyn et al. 1982; Moschovakis and Karabelas 1985). A rostral efferent pathway projects to the pretectal and thalamic regions, a medial pathway projects to spinal afferent cell groups in the medial brainstem tegmentum, and a caudal efferent pathway projects through the lateral aspects of the ipsilateral brainstem tegmentum. The similarity of these pathways in such disparate species as frogs, snakes, lizards, owls, and cats motivates the hypothesis that species-specific variations in motor behavior, including those as dramatically different as flying, walking and swimming, are not reflected in differences in the general organization of the tecto-tegmento-spinal pathways. In the barn owl, ascending auditory pathways are disproportionately large compared with homologous structures in other vertebrates. Given this conspicuous hypertrophy of sensory nuclei, we expected that effector systems specialized for generating head saccades would be similarly hypertrophied. However, we failed to observe any significant differences from other species with regard to (1) the particular cell groups that project to the spinal cord, (2) their location in the reticular formation, or (3) the density or number of cells in these nuclei.

Involvement of the tecto-tegmento-spinal pathway in orienting movements

The absence of a substantial direct tectospinal projection combined with the existence of a strong indirect tectospinal projection (established by the medial efferent pathway) focuses attention on the targets of the medial pathway as those involved in orienting movements. Recent reports in various species indicate that, between the optic tectum and the spinal cord, a major transformation in the representation of orienting movements occurs (for reviews: Grobstein 1988; Masino 1992). In the tectum, movement direction is encoded topographically. In contrast, at tegmental levels, separate cell groups in the projection field of the medial efferent pathway represent specifically vertical (Büttner et al. 1977; Masino and Knudsen 1992) or horizontal (Keller 1978; Masino and Grobstein 1989a; Masino and Knudsen 1992) components of orienting movement. As such, one likely function of the medial efferent pathway is to transform the topographically coded motor information in the tectum into a Cartesian vector representation in the tegmentum (Masino and Knudsen 1992).

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