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Primary Projections of the Trigeminal Nerve in Two Species of Sturgeon: *Acipenser oxyrhynchus* and *Scaphirhynchus platorynchus*

JOHN G. NEW AND R. GLENN NORTHCUTT
Department of Biology, Wesleyan University, Middletown, Connecticut 06457 and Division of Biological Sciences, The University of Michigan, Ann Arbor, Michigan 48109

ABSTRACT Horseradish peroxidase histochemical studies of afferent and efferent projections of the trigeminal nerve in two species of chondrostean fishes revealed medial, descending and ascending projections. Entering fibers of the trigeminal sensory root project medially to terminate in the medial trigeminal nucleus, located along the medial wall of the rostral medulla. Other entering sensory fibers turn caudally within the medulla, forming the trigeminal spinal tract, and terminate within the descending trigeminal nucleus. The descending trigeminal nucleus consists of dorsal (DTNd) and ventral (DTNv) components. Fibers of the trigeminal spinal tract descend through the lateral alar medulla and into the dorsolateral cervical spinal cord. Fibers exit the spinal tract throughout its length, projecting to the ventral descending trigeminal nucleus (DTNv) in the medulla and to the funicular nucleus at the obex. Retrograde transport of HRP through sensory root fibers also revealed an ascending bundle of fibers that constitutes the neurites of the mesencephalic trigeminal nucleus, cell bodies of which are located in the rostral optic tectum. Retrograde transport of HRP through motor root fibers labeled ipsilateral cells of the trigeminal motor nucleus, located in the rostral branchiomeric motor column.

The trigeminal nerve in the sturgeons and paddlefishes, (superorder Chondrostei), like that in agnathans and other anamniotic gnathostomes, consists of maxillomandibular branches and an ophthalmic (profundus) branch which convey somatosensory information to the medulla. The trigeminal nerve complex also possesses a motor component, which leaves the medulla as a separate rootlet and innervates the musculature of the mandibular arch. The trigeminal complex of chondrosteans is somewhat different from that of most anamniotes in that the maxillary and mandibular rami form two distinct branches directly distal to the semilunar (Gasserian) ganglion, and there is no common "maxillomandibular" root such as usually is seen in other jawed fishes (Figs. 1, 2). The profundus branch possesses a separate ganglion, but afferent fibers from this ganglion enter the main sensory trigeminal root before the root enters the medulla. The sensory fibers of the trigeminal nerve enter the lateral wall of the medulla as a compact root, with no obvious distinction between the different sensory components. The motor component of the trigeminal complex exits the wall of the medulla as a series of closely spaced rootlets located directly ventral to the entrance of the trigeminal sensory root. The number of motor rootlets varies in sturgeon, from three in *Scaphirhynchus* to five to seven in *Acipenser* (Johnston, '01, Norris, '25).

Projections of the trigeminal nerve have been studied by experimental anatomical methods in petromyzonids and teleosts (Luiten, '75; Northcutt, '79), but there exist no experimental data from the chondrosteans. In a descriptive study employing Golgi-stained material, Johnston ('01) reported that the trigeminal nerve projects to the funicular nucleus at the level of the obex via a "trigeminal spinal tract" situated at the lateral surface of the medulla. A further, descending projection also was observed to extend caudally through the medulla, medial to the

Abbreviations

ALLN, Anterior lateral line nerve
Cb, Corpus of the cerebellum
CC, Cerebellar crest
CON, Caudal octavolateralis nucleus
DG, Dorsal granular nucleus of the cerebellum
DON, Dorsal octavolateralis nucleus
DTNd, Descending trigeminal nucleus pars dorsalis
DTNv, Descending trigeminal nucleus pars ventralis
EGd, Eminentia granularis pars dorsalis
EGl, Eminentia granularis pars lateralis
EGr, Eminentia granularis pars rostralis
FR, Fasciculus retroflexus
FN, Funicular nucleus
Gg, Gasserian (semilunar) ganglion
IL, Inferior lobe of the hypothalamus
IRF, Inferior reticular formation
LL, Lateral lobule of the cerebellum
Mes, Cells of the mesencephalic trigeminal nucleus
MGN, Marginal granular nucleus
MLF, Medial longitudinal fasciculus
MON, Medial octavolateralis nucleus
MRF, Medial reticular formation
MTN, Medial trigeminal nucleus
mVn, Motor rootlet of trigeminal nerve
ob, Olfactory bulb
OET, Efferent tract of octavolateralis nuclei
OT, Optic tectum
PC, Posterior commissure
pg, Ganglion of the profundus nerve
PLLN, Posterior lateral line nerve
PN, Posterior nucleus
rALLNd, Dorsal root of the anterior lateral line nerve
rALLNv, Ventral root of the anterior lateral line nerve
rmd, mandibular ramus of the trigeminal nerve
rmV, Motor root of the trigeminal nerve
rmx, Maxillary ramus of the trigeminal nerve
rprof, profundus ramus of the trigeminal nerve
rsV, Sensory root of the trigeminal nerve
rVII, Root of the facial nerve
SpV, Spinal trigeminal tract
TC, Tectal commissure
Te, Telencephalon
TL, Torus longitudinalis
TS, Torus semicircularis
UL, Upper leaf of the marginal granular nucleus
Va, Valvula of the cerebellum
VG, Ventral granular nucleus of the cerebellum
VF, Valvular fissure
VL, Vagal lobe
II, Optic nerve
IV, Fourth ventricle
V, Trigeminal nerve
VII, Facial nerve
VIII, Otic nerve
IX, Glossopharyngeal nerve
X, Vagus nerve

spinal tract. Johnston reported this projection to be greater in cross-sectional area than the spinal tract, but less dense, and mingling with cells of the octavolateralis area. The ascending projections noted by Johnston consisted of projections to a median trigeminal nucleus at the rostral border of the medulla and a projection to the corpus of the cerebellum. Similar projections have been described by Larsell ('67) and Nieuwenhuys ('67).

This study examines the primary projections of the trigeminal nerve by experimental anatomical methods in two species of chondrostean fishes, *Scaphirhynchus platorynchus* and *Acipenser oxyrhynchus*.

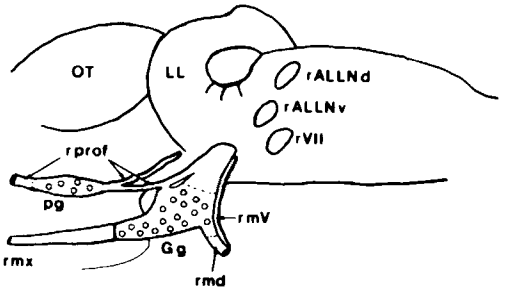

Fig. 1. Lateral reconstruction of the anterior medulla in *Scaphirhynchus*, illustrating the entrance of the sensory and motor roots of the trigeminal nerve. Modified from Norris ('25).

MATERIALS AND METHODS

Mature specimens of the shovelnose sturgeon, *Scaphirhynchus platorynchus*, weighing 106–279 gm, were obtained from the Mississippi River in the vicinity of Guttenberg, Iowa. Two juvenile specimens of the Atlantic sturgeon, *Acipenser oxyrhynchus*, were obtained by otter trawl from the Hudson River in the vicinity of Kingston, New York. The fish were maintained in large, freshwater aquaria at approximately 14°C for at least 1 week prior to surgery.

Projections of the trigeminal nerves were determined by anterograde and retrograde axonal transport of horseradish peroxidase

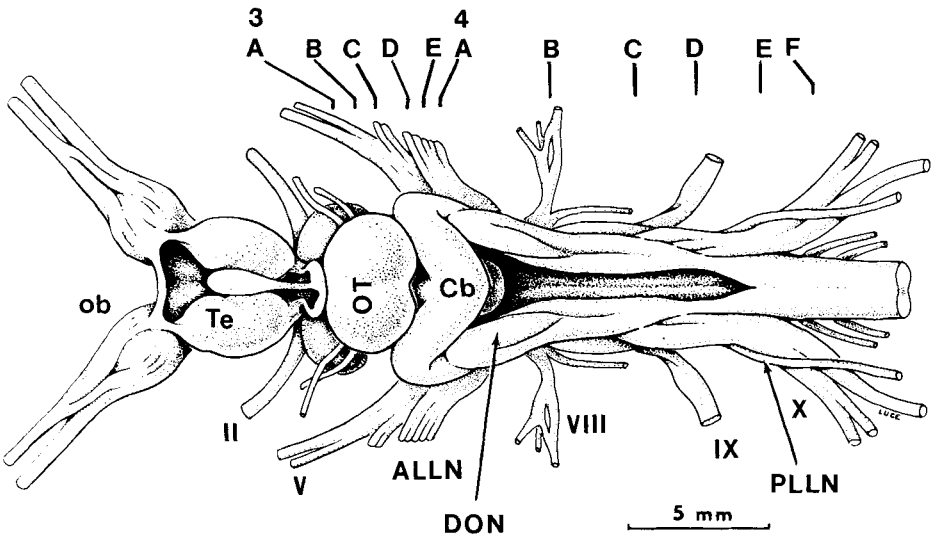

Fig. 2. Dorsal view of the brain of *Scaphirhynchus*, illustrating the position of the trigeminal nerve in relation to the other cranial nerves. The lettered bars indicate the levels at which transverse sections were illustrated in Figures 3 and 4.

(HRP) (Sigma Type VI). Sturgeon were anesthetized by immersion in a 0.025% solution of tricaine methanesulfonate (MS-222) (Sigma Type VI). The sensory or motor roots of the trigeminal nerve then were exposed and unilaterally transected proximal to the ganglion. A pledget of Gelfoam saturated with 40% HRP was placed proximal to the site of transection. The wound was then packed with Gelfoam and sealed with dental acrylic. The sensory root of the trigeminal was transected in four specimens of *Scaphirhynchus* and one specimen of *Acipenser*. In one specimen of *Scaphirhynchus*, both sensory and motor roots were transected. The motor root alone was transected in one specimen of *Acipenser* and *Scaphirhynchus*.

After survival times of 6–10 days, the sturgeon were reanesthetized and perfused transcardially with cold phosphate buffer (pH 7.4), followed by 2% glutaraldehyde in phosphate buffer. The brains then were removed and fixed for several hours in a phosphate buffer solution of 2% glutaraldehyde and 20% sucrose. Brains were embedded in a sucrose gelatin solution (30% sucrose, 10% gelatin) and the blocked brains were fixed for several hours in the glutaraldehyde-sucrose buffer solution. Transverse sections were cut on a freezing microtome at 30–40 μm and stored in cold phosphate buffer.

Sections were processed within 2–3 days by the Mesulam HRP protocol (Mesulam, '78) using either 3,3′, 5,5′ tetramethyl benzidine (TMB) or o-dianisidine dihydrochloride (DIA) (Sigma Type VI). After processing, the sections were mounted on chrome-alum slides and lightly counterstained (2 minutes) with neutral red. The location and charting of specific nuclei within the brain were accomplished with the aid of normal *Scaphirhynchus* and *Acipenser* brains, serially mounted, and stained by the Bodian and cresyl violet methods (Humason, '79).

RESULTS

Comparison of normal and experimental material from the brains of *Scaphirhynchus* and *Acipenser* demonstrated no significant differences in organization of the hindbrain, or in projections of the trigeminal nerve in the two sturgeon species. Examination of transverse sections of the HRP-labeled trigeminal cases revealed anterograde labeling of trigeminal sensory projections as well as retrograde labeling of neurons efferent to the trigeminal nerve. The sensory projections of the trigeminal nerve consist of medial and

descending components. Retrograde labeling of fibers and cells of the mesencephalic trigeminal nucleus also was observed in sensory root transection cases.

Projections of the sensory root of the trigeminal nerve

Medial sensory projections

Upon entering the lateral wall of the hindbrain, a large number of sensory fibers course medially and terminate within an ipsilateral nucleus that lies adjacent to the medial wall of the medulla (Figs. 3D–E, 4A, 5). This nucleus, the medial trigeminal nucleus (MTN), consists of small and medium-sized fusiform and multipolar cells and extends from the level of entrance of the trigeminal nerve rostrally to the level of the caudal cerebellum. Some labeled afferent fibers ascend parallel to the MTN and then turn medially and enter the MTN at a level rostral to the entrance of the trigeminal nerve (Fig. 3E).

Descending trigeminal sensory projections

Upon entering the medulla, the majority of trigeminal afferent fibers turn caudally and descend through the ipsilateral alar medulla to the dorsolateral cervical spinal cord. The descending projections consist of two major fascicles: the fibers of the trigeminal spinal tract, and fibers projecting to, and terminating in, the descending trigeminal nucleus. The descending trigeminal nucleus itself consists of two major components—the pars dorsalis (DTNd) and the pars ventralis (DTNv).

The trigeminal spinal tract comprises a dense bundle of afferent fibers that descend through the alar medulla and into the cervical spinal cord (Figs. 4B–F, 7, 8). The spinal tract is positioned ventral to the nuclei of the octavolateralis region and ventral to the entering roots of the glossopharyngeal (IXth) (Figs. 4C–E, 7) and vagus (Xth) nerves. Along its length, fibers leave the spinal tract to terminate in the DTNv; however, no fibers appear to enter the DTNd from the spinal tract. Caudal to the level of the obex, fibers leave the spinal tract to terminate densely in the lateral portion of the funicular nucleus, a region of dense neuropil and neurons in the dorsal cervical spinal cord (Figs. 4E, 8). Scattered fibers of the spinal tract continue to descend through the cervical spinal cord, extending caudal to the first spinal segment (Fig. 4F) and terminating in the dorsolateral region of the spinal cord.

The pars dorsalis (DTNd) and the pars ventralis (DTNv) of the descending trigeminal nucleus lie medial to the spinal tract and directly ventral to the nuclei of the octavolateralis region (Figs. 4B–E, 7). The DTNd extends caudally from the entrance of the trigeminal sensory root to a level just caudal to the entrance of the glossopharyngeal (IXth) nerve (Figs. 4C, 7B). The DTNd is located immediately dorsal to the DTNv in the rostral medulla, however the distance between them increases caudally. The caudal DTNd is positioned dorsal to the entering glossopharyngeal root, whereas the DTNv is located ventral to the entering root fibers (Figs. 4C,7B).

Similarly, the DTNv can be traced from the entrance of the trigeminal sensory root, although it extends somewhat more caudally than does the DTNd. The DTNv terminates at a point just rostral to the obex (Fig. 4D). The DTNv is located immediately ventral to the DTNd in the rostral medulla but diverges at the level of entrance of the glossopharyngeal nerve as described above.

The mesencephalic trigeminal nucleus

Examination of HRP-stained sections also revealed a collection of fibers entering the medulla via the sensory root of the trigeminal nerve and ascending into the midbrain. These fibers extend through the MTN and course ventrolateral to the lateral cerebellum (Fig. 3A–C), where they turn dorsally, pass through the torus semicircularis, and enter the rostral optic tectum. These fibers are the retrogradely labeled axons of large pseudounipolar cells located in the periventricular zone of the optic tectum (Figs. 3A,B, 6). The majority of these cells are located ipsilateral to the examined nerve, but occasionally, fibers cross the tectal commissure and arise from labeled cells in the contralateral tectum (Figs. 3B, 6B). The ascending fibers of this mesencephalic trigeminal tract do not seem to have any terminal field within the corpus of the cerebellum or the lateral tegmentum.

Efferent connections of the trigeminal nerve

With the exception of the mesencephalic trigeminal nucleus, no retrograde labeling of neuronal somata was observed after transection of the sensory root of the trigeminal nerve. However, after transection of the motor root of the trigeminal nerve, large multi-

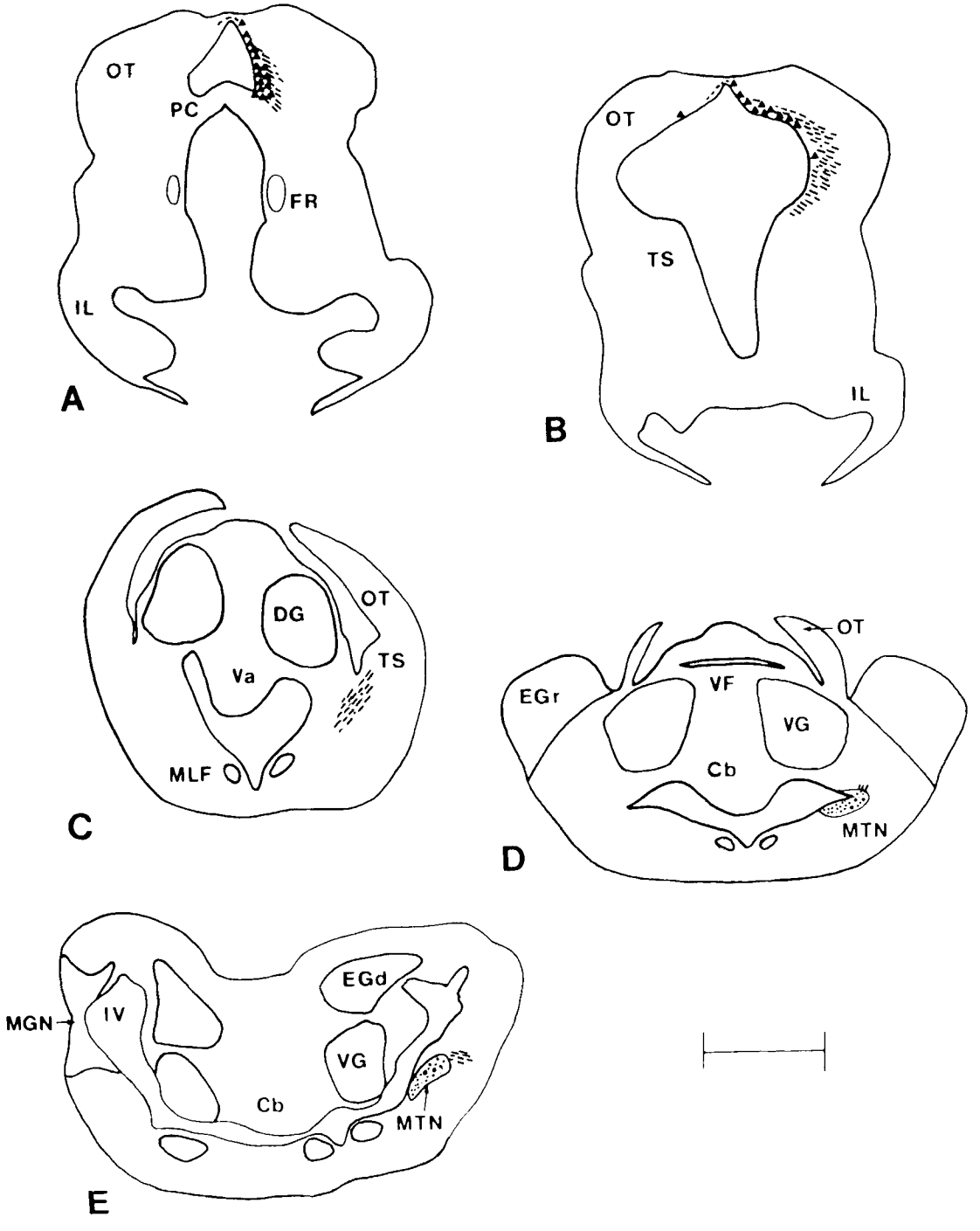

Fig. 3. Transverse sections through the cerebellum and mesencephalon of *Scaphirhynchus* illustrating projections of the trigeminal nerve as demonstrated by HRP histochemistry. Dashed lines and large dots indicate nerve fibers; small dots indicate terminal fields. Triangles represent labeled cell bodies.

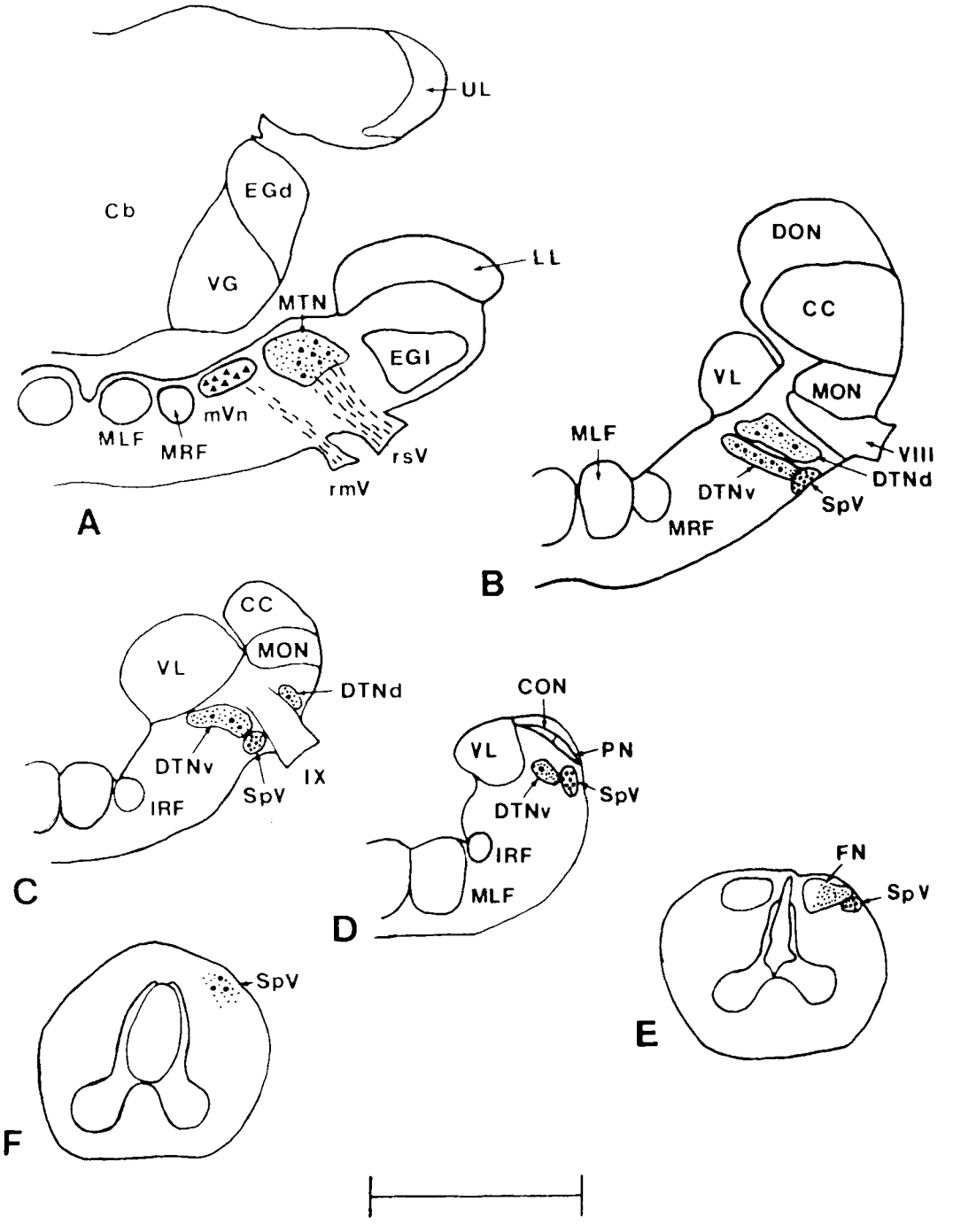

Fig. 4. Transverse sections through the medulla and cervical spinal cord of *Scaphirhynchus* illustrating projections of the trigeminal nerve as demonstrated by HRP histochemistry. Dashed lines and large dots indicate nerve fibers; small dots indicate terminal fields. Triangles represent labeled cell bodies. Scale equals 1 mm.

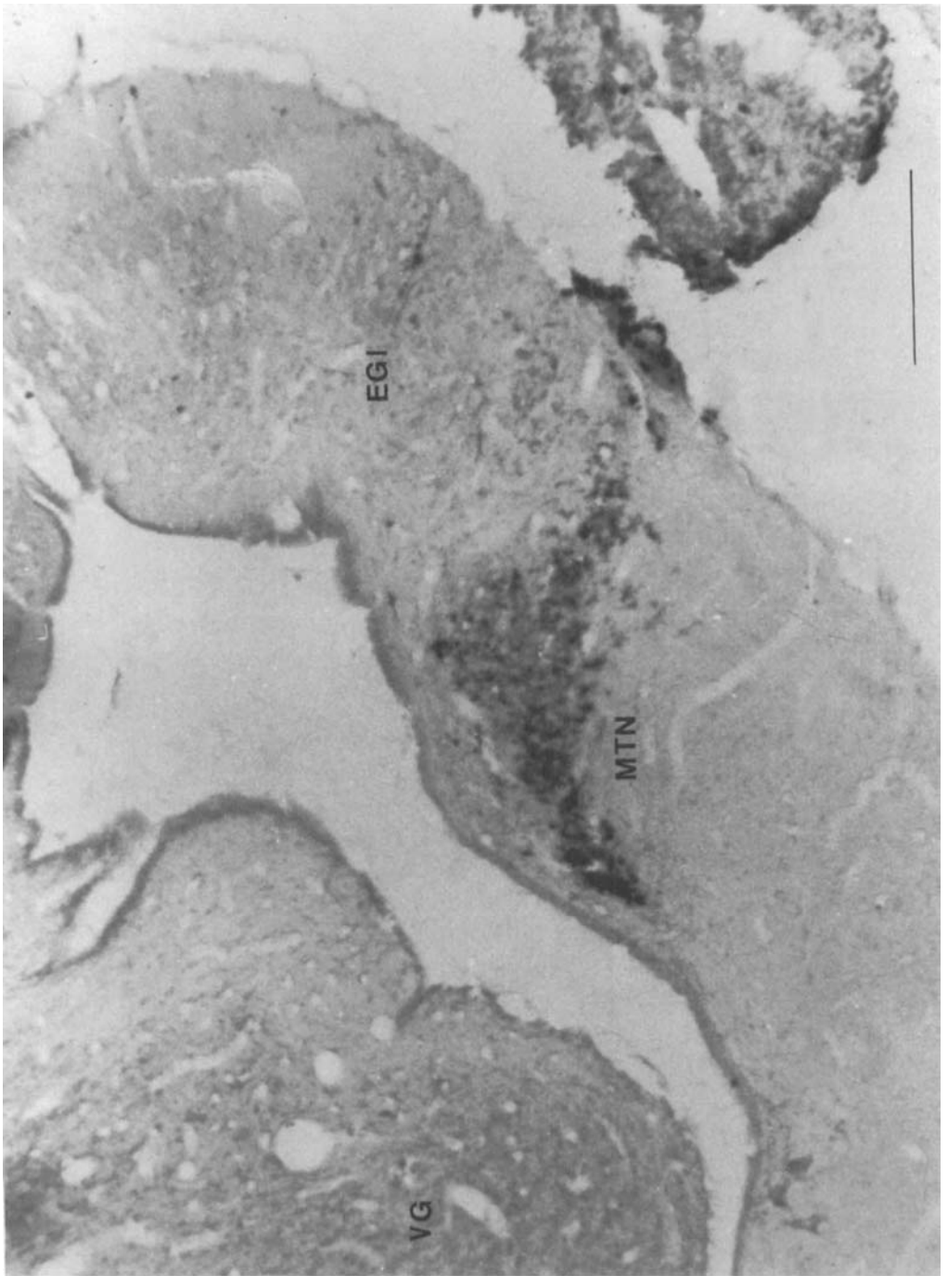

Fig. 5. Transverse section through the rostral medulla of *Scaphirhynchus* at approximately the level of Figure 3E, indicating HRP-labeled fibers terminating within the medial trigeminal nucleus. Scale bar = 0.25 mm.

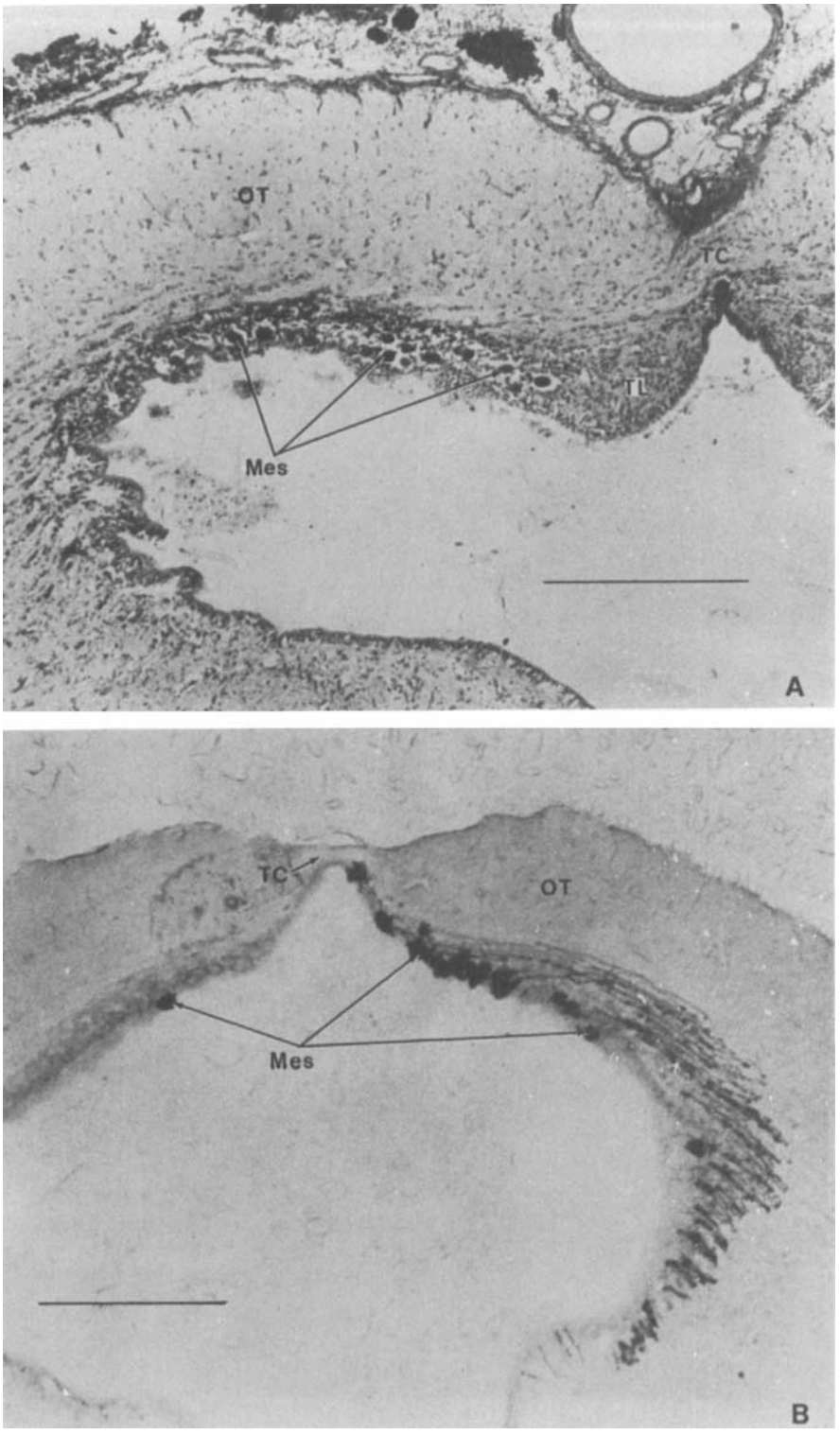

Fig. 6. A. Cresyl-violet-stained transverse section through the rostral optic tectum of *Acipenser*, indicating position of the cell bodies of the mesencephalic trigeminal nucleus. B. Transverse section through the rostral optic tectum of *Scaphirhynchus* indicating HRP-labeled cells of the mesencephalic trigeminal nucleus. Note labeled contralateral cell. Scale equals 0.5 mm.

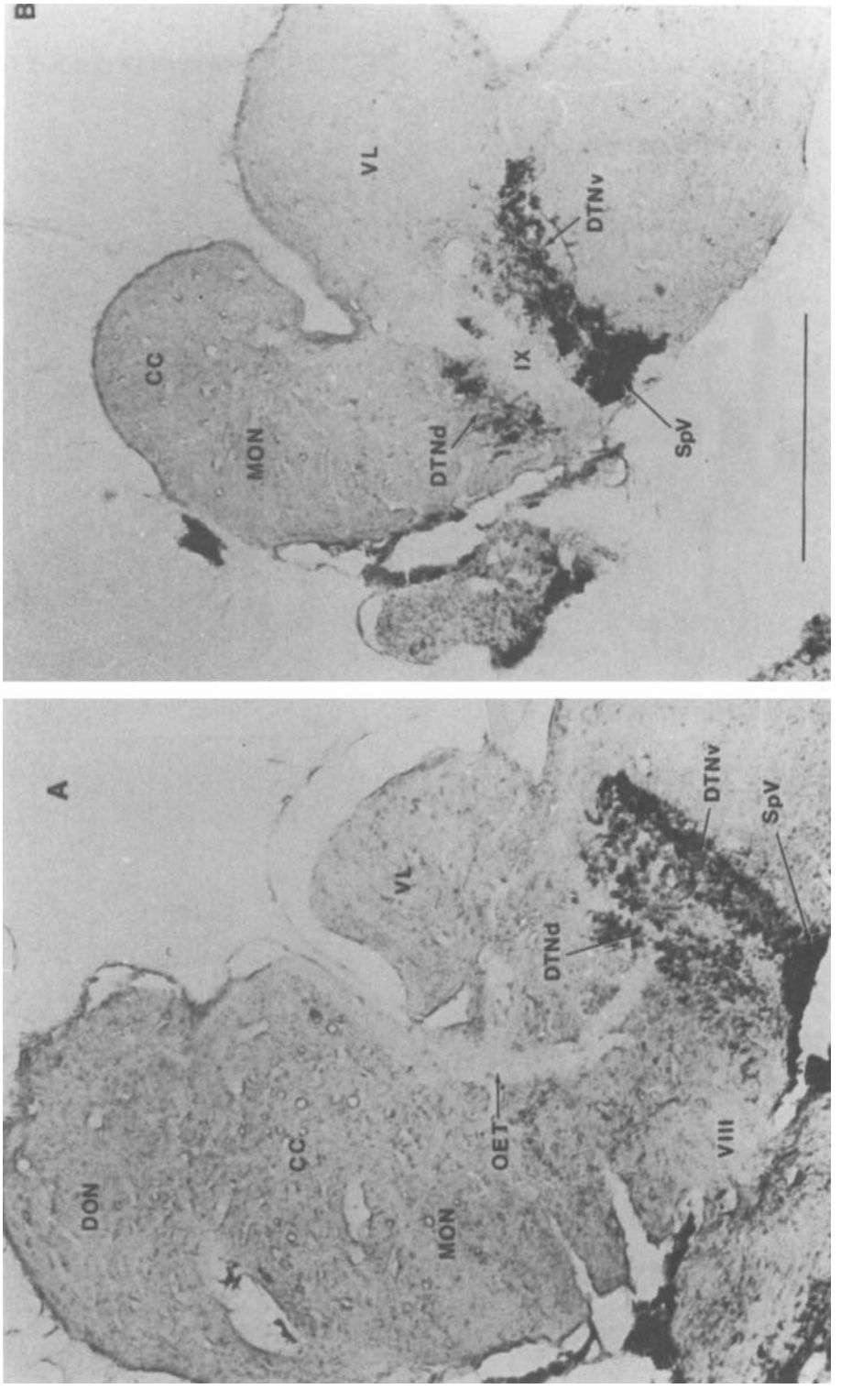

Fig. 7. A. Transverse section through the medulla of *Scaphirhynchus*, indicating HRP-labeled descending trigeminal projections at approximately the level of Figure 4B. Scale equals 0.5 mm. B. Transverse section through the medulla of *Scaphirhynchus*, indicating HRP-labeled descending trigeminal projections at approximately the level of Figure 4C. Scale equals 0.5 mm.

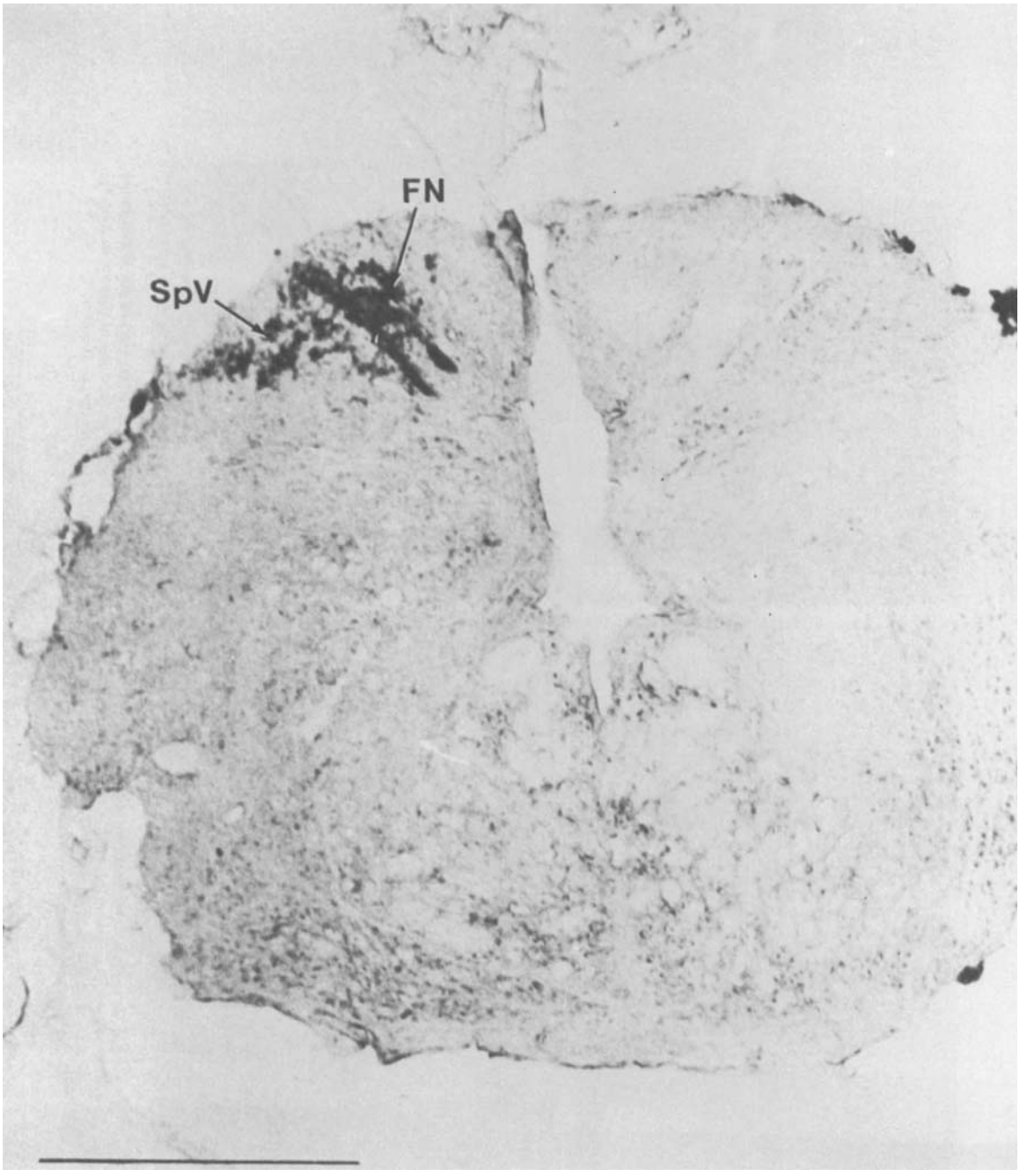

Fig. 8. Transverse section through the rostral cervical spinal cord indicating descending trigeminal projections at approximately the level of Figure 4F in *Scaphirhynchus*. Scale equals 0.5 mm.

polar neurons in the rostral portion of the branchiomeric motor column were labeled retrogradely with HRP, and fibers could be observed passing from these cells out of the medulla via the motor root of the trigeminal nerve (Fig. 4A). Only cells ipsilateral to the nerve were labeled with HRP, and these cells comprise the motor nucleus of the trigeminal nerve described in several studies (Larsell, '67; Nieuwenhuys, '83; New and Northcutt, '84).

DISCUSSION

The present results confirm and extend previous nonexperimental anatomical observations of trigeminal projections in sturgeon. Johnston ('01) reported trigeminal sensory projections to a "median trigeminal nucleus" lying along the medial wall of the rostral medulla, and our experimental results confirm his observation. A similar nucleus has been described in holosteans and teleosts (Woodburne, '36; Luiten, '75) and elasmobranchs (Northcutt, '78; Nieuwenhuys, '83). Woodburne ('36) termed this nucleus the sensory trigeminal nucleus, and described it in *Amia*, *Salmo*, and *Cyprinus*, while Luiten ('75) described it as cell group I in a teleost, *Cyprinus*. In elasmobranchs, it has been described as the principal sensory trigeminal nucleus in *Raja* (Northcutt, '78), and as nucleus C in *Scyliorhinus* (Smeets and Nieuwenhuys, '76). Whether any of these nuclei are homologous to the main sensory trigeminal nucleus of mammals is unknown and experimental anatomical elucidation of the efferent connections of these nuclei are needed to assess possible homologies.

The organization of descending trigeminal nuclei also agrees to a certain extent with Johnston's observations. Johnston described a spinal trigeminal tract, but did not observe projections caudal to the funicular nucleus. The HRP material clearly demonstrates projections of the spinal tract into the dorsolateral cervical spinal cord. Similar spinal projections have also been described in lampreys (Northcutt, '79), elasmobranchs (Smeets et al., '83), and teleosts (Luiten, '75). Two studies (Johnston, '01; Larsell, '67) also noted that fibers of the IXth and Xth cranial nerves project into the trigeminal spinal tract. Preliminary experiments in *Scaphirhynchus* indicate that these projections do exist (New and Northcutt, unpublished observations). In addition to the spinal tract projections, sensory trigeminal projections deep to the spinal tract have been described in other anamniotes: in *Amia* (Woodburne'36) and in teleosts (Ariens Kappers et al., '36; Luiten, '75), as well as in chondrosteans (Johnston '01; Hocke Hoogenboom '29). Ariens Kappers et al. described dorsal and ventral divisions of the descending trigeminal nucleus in *Lophius*, and stated that the dorsal division contained descending fibers from the maxillomandibular root, whereas the ventral division contained descending fibers from the ophthalmic root. Although we have not reported herein on the individual projections of the separate rami of the trigeminal nerve, preliminary experiments in the paddlefish, *Polyodon* (New, unpublished observations), indicate that the maxillomandibular root appears to contribute fibers only to the spinal tract and DTNv in chondrosteans. Luiten ('75) also reported descending trigeminal projections to a group of cells ventrolateral to the motor nucleus of the vagus nerve. This projection was not observed in sturgeon.

A mesencephalic trigeminal nucleus has been described in most classes of vertebrates, with the exception of the agnathans, which do not appear to possess one (Hocke Hoogenboom, '29; Woodburne, '36; Witkovsky and Roberts, '75; Smeets and Nieuwenhuys, '76; Northcutt, '79; McDonell, '80). Johnston ('01) did not recognize a mesencephalic trigeminal nucleus in *Acipenser*, but did note a magnocellar nucleus in the rostral optic tectum, cells of which he was unable to stain by the Golgi method. These cells were retrogradely labeled with HRP in the present study and constitute the mesencephalic trigeminal nucleus. Our experiments indicate that the fibers exit the medulla via the sensory root of the trigeminal nerve, as has been reported in *Amia*, rather than by the motor root, as has been observed in *Salmo* (Woodburne, '36).

Retrograde transport of HRP into the motor root of the trigeminal nerve revealed a prominent collection of labeled cells in the ipsilateral rostral branchiomeric motor column. This group of cells was described previously as the motor nucleus of the trigeminal nerve, and the results described here support that conclusion.

The projections of the trigeminal nerve in sturgeon greatly resemble the projections observed in other anamniotic gnathostomes. Of particular interest is the dorsoventral divi-

sion of the descending trigeminal nucleus. Further studies are necessary to determine whether this division reflects a somatotopic segregation of afferent fibers from the different branches of the trigeminal nerve.

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