

Motor Nuclei of Nerves Innervating the Tongue and Hypoglossal Musculature in a Caecilian (Amphibia: Gymnophiona), as Revealed by HRP Transport

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ABSTRACT

The organization of the motor nuclei of the glossopharyngeal, vagal, occipital, first spinal and second spinal nerves of *Typhlonectes natans* (Amphibia: Gymnophiona: Caeciliidae: Typhlonectinae) was studied by using horseradish peroxidase reaction staining. Each nucleus has discrete patterns of cytoarchitecture and of topography. Nuclei are elongate and some overlap anteroposteriorly. The brainstem is elongate, with no distinct demarcation of brainstem from spinal cord. The occipital nerve emerges through a separate foramen from that for the vagus and glossopharyngeal nerves in the species studied, is distinct from both, and its nucleus is more similar to spinal nuclei in cytoarchitecture. The occipital nerve fuses with spinal nerves 1 and 2 to contribute to the hypoglossal trunk. A spinal accessory nerve is absent. © 1996 Wiley-Liss, Inc.

Indexing terms: brainstem, ramus hypoglossus, occipital nerve

The neuroanatomy of the gymnophione amphibians (caecilians) has received little attention, especially in comparison to the extensive work published on salamanders and frogs. Waldschmidt (1887) briefly described the anatomy of the brain and cranial nerves in four species of caecilians; Burckhardt (1891) compared the brains of the salamander *Triturus* and the caecilian *Ichthyophis*. Norris and Hughes (1918) examined the cranial and anterior spinal nerves of several species of caecilians; Kuhlenbeck (1922) described the brain, concentrating on *Siphonops annulatus*. Laubmann (1927) examined morphogenesis of the brain of *Hypogeophis*, and Krabbe (1962) compared gross brain development in three species of salamanders and three of caecilians, each in different families. While none of these authors considered details of brain organization in caecilians, their studies are the point of departure for our work.

Recent studies on the motor nuclei of frogs (Szekely, 1976; Matesz and Szekely, 1977, 1978; Stuesse et al., 1983, 1984; Nikundiwe et al., 1982; Nikundiwe and Nieuwenhuys, 1983; Oka et al., 1987) and salamanders (Roth and Wake, 1985; Wake et al., 1988), and both (Szekely and Matesz, 1993), revealed differences in the organization of motor nuclei (number of motor columns, segregation of motor nuclei) between members of the two orders. Frogs differ from salamanders in having a well-expressed topographic segregation of motor nuclei in the anterior medulla

oblongata. Motor nuclei of spinal nerves are constituted of medial and lateral motor columns. In salamanders, there is extensive overlap of adjacent motor nuclei. Spinal nuclei of bolitoglossine salamanders are constituted of only a medial motor column, (Roth and Wake, 1985; Roth et al., 1988; Wake et al., 1988). Caecilians also have a more reduced brain morphology than do frogs. However, studies on the tectum mesencephali in caecilians (Schmidt and Wake, 1991) suggest that these animals represent an "intermediate" degree of morphological complexity, between that of frogs and salamanders. Modes of feeding and locomotion in caecilians are markedly different from those of salamanders and frogs. However, to date, nothing is known about the organization of the caecilian brainstem and the cervical spinal cord and its relation to feeding and locomotion in caecilians. Therefore, on strict functionalist grounds, differences in the neuroanatomy of medullary control regions are expected.

The aim of this study is to examine the topology and cytoarchitecture of motor nuclei in one species of caecilian, *Typhlonectes natans*, and to make a general comparison

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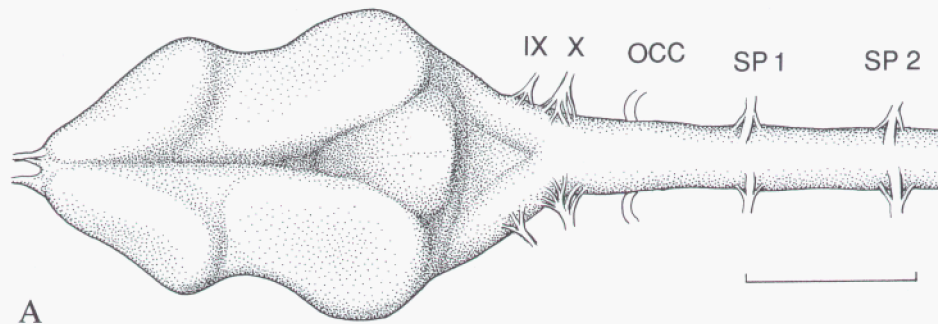
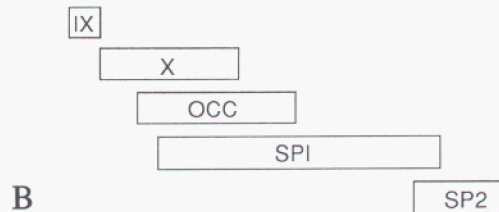


Fig. 1. Dorsal view of the brain of *Typhlonectes natans*, illustrating the conformation of the posterior cranial and anterior spinal nerves (A), and the extent and alignment of the motor nuclei of those nerves in the brainstem (B). IX, glossopharyngeal nerve; X, vagus nerve; OCC, occipital nerve; SP1, first spinal nerve; SP2, second spinal nerve. Scale bar = 2 mm.



with the condition in salamanders and frogs to determine whether differences in function are correlated with differences in neuroanatomical organization. This study establishes a baseline for further comparative work on the neuroanatomy of caecilians, and elucidates several new questions that we expect to consider through experiments employing a wider range of techniques.

MATERIALS AND METHODS

The brains of 17 *Typhlonectes natans* (Amphibia: Gymnophiona: Caeciliidae: Typhlonectinae, Hedges et al., 1993) were prepared for examination of the posterior cranial and anterior spinal nerves, and their motor nuclei (see Figs. 1, 2). Twelve animals were used to stain cranial nerves IX, X, the occipital nerve, and spinal nerves 1 and 2 by the horseradish peroxidase (HRP) method. Five animals were prepared as wholemount, cleared and Sudan black B-stained specimens (according to modifications of the techniques of Filipinski and Wilson, 1984 and Nishikawa, 1987; see Wake, 1992).

Under tricaine methane sulfonate (MS 222) anaesthesia (1:100 w/v solution), various combinations of the glossopharyngeus (IX), vagus (X), occipitalis, spinal 1, and spinal 2 were severed on the left and right sides of the animals. Nerves were identified according to Wake (1992); operational definitions of the occipital and the spinal accessory nerves are provided there and in the Discussion. HRP tracing and reactions were performed according to Fritzsche et al. (1984). Brains were then dehydrated in a series of 4 ethanols (70–100%) for 30 minutes, then cleared and stored in 100% methyl salicylate. In order to make serial reconstructions of the motor nuclei and neurons, wholemounts were returned to alcohol, embedded in Epon, and cut at 60 μm . Brains so prepared were compared with five specimens macerated in trypsin, bulk-stained with Sudan black B and alizarin red S, cleared in glycerine, and stored in 100% glycerine to which thymol crystals were added to prevent fungal growth (see Wake, 1992, for protocol and comparative material). Sudan black B staining of intact animals permits examination of peripheral nerves and their

branches. HRP-reacted brains were also compared to serially sectioned, Palmgren's stained heads in order to study peripheral nerve patterns and central nervous system (CNS) tracts.

RESULTS

External morphology

The caecilian brainstem contrasts markedly with that of frogs and salamanders in having a sharp ventral bend at the level of the posterior end of the greatly enlarged hypothalamus, similar to that in many mammals. The bend distorts the brainstem.

Motor pathways and nuclei

Glossopharyngeus (IX). There are two distinct but very small rootlets that orient rather sharply posteriorly when they enter the brainstem. The motor rootlets of the glossopharyngeus are well-separated from and cranial to those of the vagus. The motor nucleus of the glossopharyngeus is well-separated from the motor nucleus of the vagus (Fig. 1). It is small and compact (Figs. 1, 3B), and lies entirely in front of the obex. Motor neurons of the glossopharyngeus are found in the periventricular zone.

Vagus (X). The vagus enters the brain by way of three motor branches, each composed of two rootlets. The motor nucleus of the vagus is relatively large (Figs. 1, 2, 4A,B) and extends ventrolaterally from a point between the entrances of the glossopharyngeus and the vagus in front of the obex posteriorly to a point between the entrances of the occipital nerve and the first spinal nerve, well behind the obex (Fig. 4). Cells lying in the dorsal part of the ventrolateral column of motor neurons are characterized by horizontal dendritic arborizations that extend laterally and anteriorly, and constitute a lateral neuropil (Fig. 4D).

Occipitalis. The occipital nerve is exclusively motor and lacks a dorsal root. It enters the brain via two branches between the vagus and spinal 1, somewhat nearer the vagus. The anterior branch consists of two rootlets and the posterior branch of four rootlets. The motor nucleus ex-

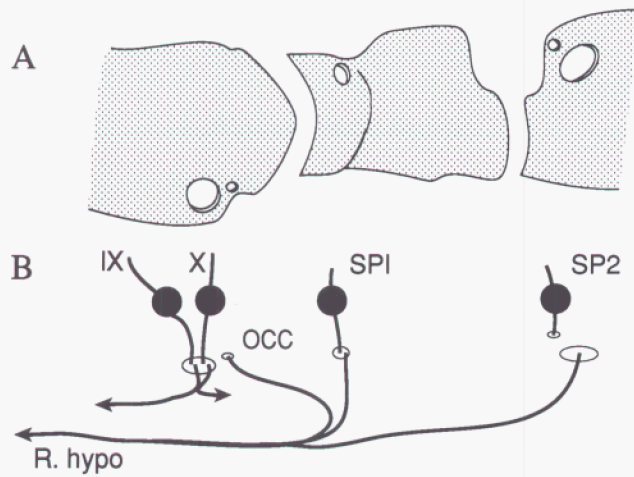


Fig. 2. Schematic illustration of the skeletal elements (A) and peripheral nerve roots (B) associated with the posterior cranial and anterior spinal nerves. A is a drawing of the rear of the skull, the atlas, and the first spinal (second) vertebra, not drawn to scale, but to accent the foramina through which the nerves exit. Cranial nerves IX and X exit together through the large foramen in the skull, the occipital through the smaller posterior foramen. Spinal 1 exits through the foramen of the atlas; the dorsal and ventral roots of spinal 2 exit separately, the dorsal through the small anterior foramen, the ventral through the larger, more ventral foramen. B diagrams the nerves and their associations to form the ramus hypoglossus (R. hypo.; other abbreviations as in Fig. 1). Closed dark circles indicate dorsal root ganglia. Open circles indicate exit foramina. Ventral components of spinal nerves 1 and 2, and the solely ventral occipital nerve, compose the ramus hypoglossus. A and B are aligned to indicate the extents of skeletal elements and nerve root configurations relative to each other.

tends forward to the level between the entrances of the glossopharyngeal and vagal nerves and posterior to a level between the entrances of the occipital and first spinal nerves (Figs. 2, 4A,B). There is strong overlap with that of the vagus (Figs. 2, 4A). Most motor neurons of the occipitalis are found in the ventral part of the lateral medulla. Dendritic arborizations of these cells project dorsad and ventrad and appear to contact the lateral neuropil constituted by neurons of the vagus. A few cells lie medially and form a separate column, continuous with that of the medial column of more posterior nuclei. These cells are small and weakly stained; we suspect that our staining of these cells may be incomplete. The dendritic arborizations of these cells extend laterad (Fig. 4D).

Spinal 1. The first spinal nerve has distinct, large dorsal and ventral roots, and a large dorsal root ganglion. The dorsal root is widely separated from the ventral root, and is posterior to it. The motor nucleus of the first spinal nerve lies ventrolaterally. A few small pear-shaped cells are found medially, forming a medial column. The ventrolateral column extends anteriorly to overlap the posterior portion of the nuclei of the vagus and occipitalis (Figs. 2, 4B), and posteriorly to a point anterior to the entrance of the second spinal nerve. Ventrolateral motor neurons arborize dorsolaterally. There are few large multipolar cells; they extend dendrites in all directions.

Spinal 2. Motor branches enter the cord via two anterior rootlets. A few stained motor neurons lie anterior to the entrance of the motor branches (Fig. 4E). The nucleus extends anteriorly to the level of the midpoint between the

entrances of the first and second spinal nerves. Dendritic arborizations of motor neurons extend dorsad and constitute a neuropil lateral to the sensory projection of spinal 2 (Fig. 4E).

DISCUSSION

Organization of amphibian motor nuclei

There are differences in the organization of motor nuclei among members of the three orders of amphibians regarding the organization of motor columns, cytoarchitecture, and the pattern of segregation of motor nuclei (Matesz and Székely, 1978; Stuesse et al., 1983, 1984; Nikundiwe et al., 1982; Nikundiwe and Nieuwenhuys, 1983; Oka et al., 1987; Roth and Wake, 1985; Roth et al., 1988; Wake et al., 1988; Székely and Matesz, 1993; Wake, 1993). The motor nuclei of frogs are more widely separated than are those in salamanders. The organization of the brainstem in salamanders is characterized by extensive overlapping of motor nuclei. In caecilians, the internal organization of the motor nuclei (i.e., the formation of different motor columns) is more similar to that of frogs, but the pattern of overlap more closely resembles that of salamanders (compare Fig. 1 with Fig. 6 in Roth et al., 1990).

The brainstem of *Typhlonectes* is similar to that of frogs, and unlike that of salamanders, in having a clear segregation of the glossopharyngeal and vagal nuclei. However, the posterior part of the medulla is characterized by an overlapping of motor nuclei, as occurs in salamanders. We found extensive overlap among the motor nuclei of the occipitalis, the vagus, and the rostral half of the first spinal nerves. Overlap of the nuclei of the vagus and spinal 1 also has been described in salamanders (Wake et al., 1988). Neither the occipital motor nucleus nor its nerve occur in frogs or salamanders, or in amniotes.

An apparent condensation of spinal 1, spinal 2, and the hypoglossal nerves has occurred in adult anurans. Spinal 1, with dorsal and ventral roots and a ganglion, is present in larval frogs (Gaupp, 1899). A complex of nerves emerges from the first vertebra in adult frogs, including those that form the hypoglossal ramus (Stuesse et al., 1983). However, there is no ganglion, and no clear dorsal root of spinal 1. The rostral parts of the two distinct motor columns associated with these nerves overlap the posterior half of the vagal nucleus in *Bufo* (Oka et al., 1987).

Glossopharyngeal and vagal motor nuclei form a continuous (salamanders: Roth et al., 1988), partially discontinuous (caecilians), or discontinuous (frogs: Oka et al., 1987) single column of cell bodies, lying at the ventrolateral margin of the periventricular gray matter. The spinal motor nuclei include two columns, one medial and the other ventrolateral, in salamanders (except for the derived, simplified condition in bolitoglossines; Wake et al., 1988). The occipital motor nucleus in *Typhlonectes* is more similar to the more posterior spinal nuclei than to the more anterior glossopharyngeal and vagal nuclei, in that both medial and ventrolateral motor neurons are present. However, either our staining of the medial column is incomplete, or this column is constituted by very few and very small cells in *Typhlonectes*.

Fetcho (1986, 1987) suggested distinct functions for the spinal motor columns in amniotes. The medial column, which arises early in ontogeny, was thought to innervate axial musculature; the lateral column was considered re-

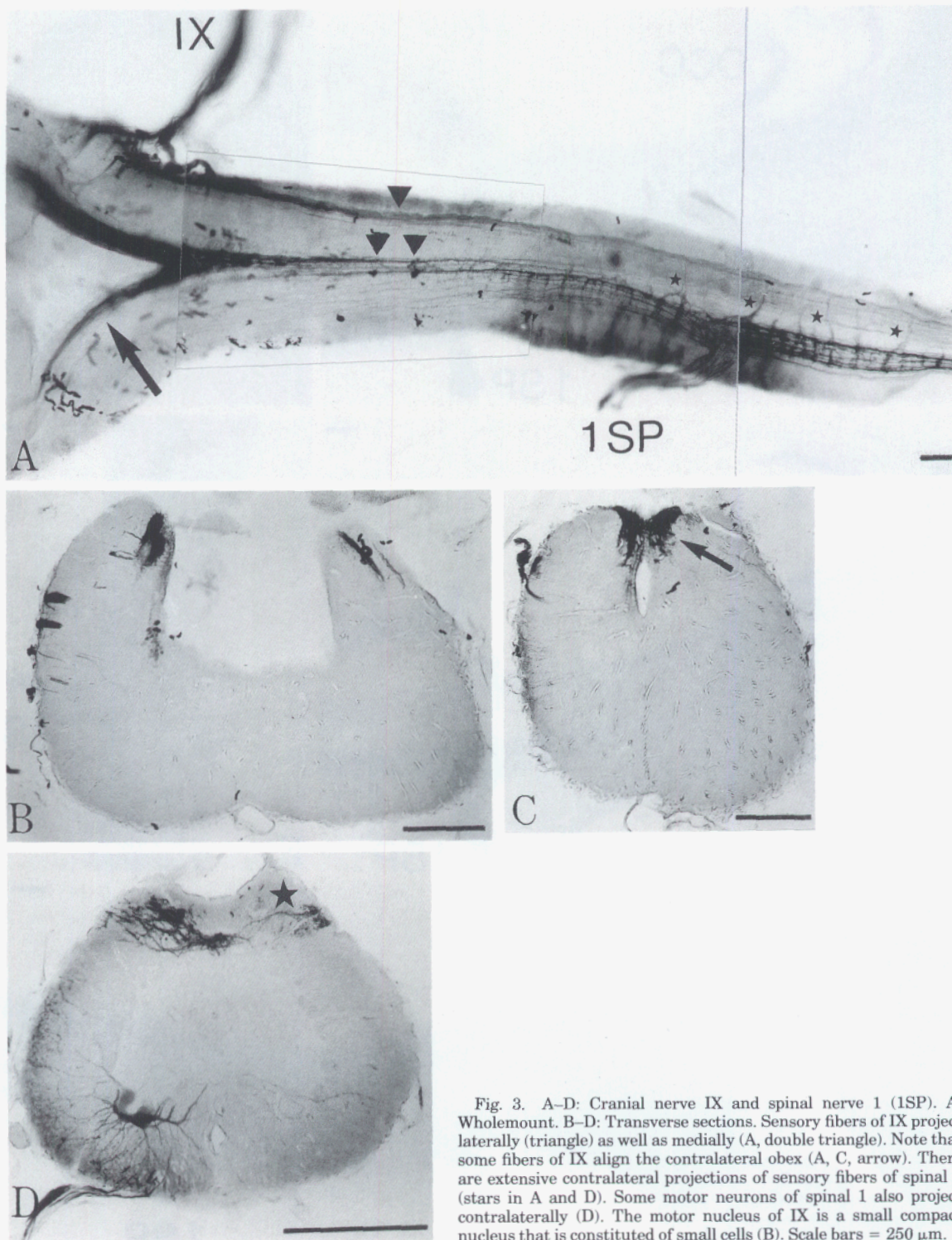


Fig. 3. A-D: Cranial nerve IX and spinal nerve 1 (1SP). A: Wholemount. B-D: Transverse sections. Sensory fibers of IX project laterally (triangle) as well as medially (A, double triangle). Note that some fibers of IX align the contralateral obex (A, C, arrow). There are extensive contralateral projections of sensory fibers of spinal 1 (stars in A and D). Some motor neurons of spinal 1 also project contralaterally (D). The motor nucleus of IX is a small compact nucleus that is constituted of small cells (B). Scale bars = 250 μ m.

lated to limb musculature. Van Mier et al. (1985) showed that development of the lateral motor column in *Xenopus* is correlated with development of the limbs. Such functions cannot be attributed to caecilians, for not only do they lack limbs, but they also have lateral columns. Axial musculature therefore must be innervated by the lateral columns. Matesz and Székely (1977) report that in *Rana esculenta*

the medial column innervates tongue musculature. Roth and Wake (1985) suggest that the lateral column in both frogs and salamanders may be involved with neck and body rather than with tongue movement. We found very few motor neurons lying medially in *Typhlonectes*, provided that the small number is not a consequence of incomplete staining. The small number of medial motor neurons may

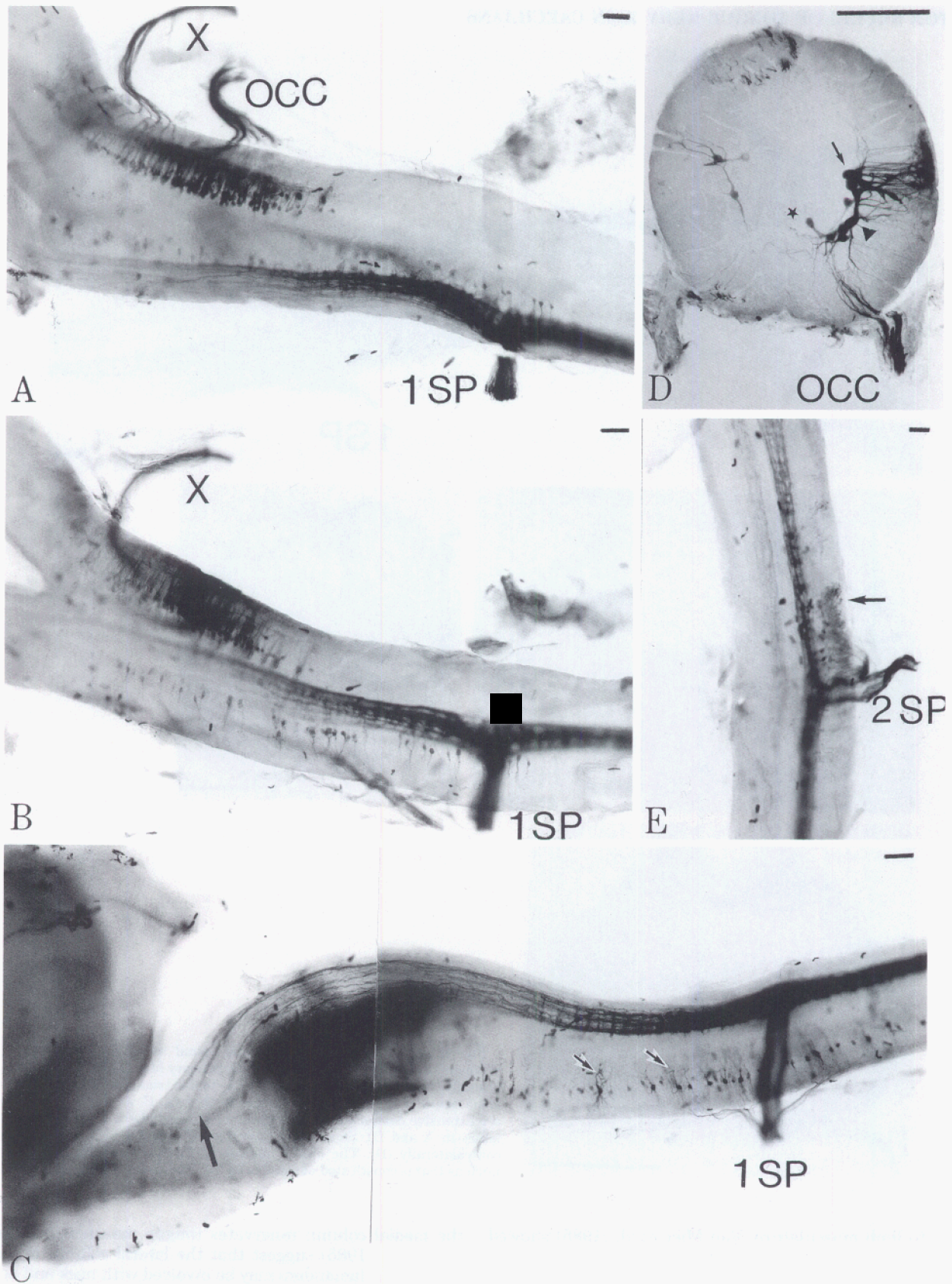


Fig. 4. A–E: Cranial nerve X (X), occipital (OCC), spinal 1 and spinal 2 (1SP, 2SP). A,B,C,E: Wholemouts. D: Transverse section of the wholemout in A at the level of X and occipital. The anterior part of the nucleus of spinal 1 and the posterior parts of the X and occipital nuclei overlap (B). In D, motor neurons are found in three positions: medial (star), ventrolateral (triangle), and in the dorsal part of the ventral horn

(arrow). We consider the latter cells part of the Xth nucleus, and the ventrolateral and medial motor neurons are occipital. Motor neurons of spinal 1 and of spinal 2 project dorsolaterally (thin arrows). Sensory projections of spinal 1 extend forward, beyond the obex (thick arrow). Scale bars = 250 μ m.

be related to a reduction of tongue components in these animals (Wake, 1992).

Occipital and/or spinal accessory nerves

The presence of an exclusively motor occipital nerve and its large nucleus in some species of caecilians long has been known (Marcus, 1910; Norris and Hughes, 1918). Wake (1992) reviewed the literature on occipital nerves, and concluded that the nerve in caecilians is appropriately so designated for several reasons (see below). The occipitalis apparently is involved only in the constitution of the ramus hypoglossus. After leaving the brain and proceeding distally, either through a common foramen with the glossopharyngeus and vagus (several other taxa; see Wake, 1992), or through its own cranial foramen (as in the species reported herein), the occipitalis fuses with spinal 1, which then joins spinal 2 (Norris and Hughes, 1918; Wake, 1992). We do not yet have anterograde staining that would identify the innervation targets of the specific components of the ramus hypoglossus.

We do not find a spinal accessory nerve in *Typhlonectes*, nor in other species prepared for study (Wake, unpublished data), nor has one been reported in the literature for caecilians. Some have questioned the identification of the occipitalis as such (personal communication to M. Wake), rather than as a spinal accessory, since the latter now has been found in salamanders (Roth et al., 1984; Roth and Wake, 1985; Wake et al., 1988) and frogs (Matesz and Székely, 1977; Oka et al., 1987; Székely and Matesz, 1993), as well as many other vertebrate taxa. Location of its motor neurons suggests that the caecilian occipitalis might resemble a spinal nerve; however, as Wake (1992, 1993a,c) discussed, the occipitalis of caecilians is not homologous to the spinal accessory nerve of salamanders. Therefore, caecilians are distinguished from their sister taxa by the presence of an occipital nerve in derived species, and by the apparent absence of a spinal accessory nerve. Consequently, the general conclusion that a spinal accessory nerve is present in all amphibians (Székely and Matesz, 1993) does not apply to caecilians, based on current information.

The constitution of spinal nerve 1 in amphibians

The first spinal nerve of adult salamanders typically is composed of several ventral rootlets, and lacks a dorsal root and ganglion. When the latter are present in embryos, they are lost in adults (Roth and Wake, 1985). Spinal 1 is strictly motor and innervates tongue musculature via the ramus hypoglossus. The second spinal nerve has dorsal and ventral roots, and a dorsal ganglion; it innervates various throat, tongue, and neck muscles, as well as contributing a branch to the brachial plexus. In frogs, spinal nerve 1 is present in tadpoles but disappears after metamorphosis (*Rana*: Gaupp, 1899), and the contribution to the ramus hypoglossus emerges from the ventral ramus of the second spinal nerve (Stuesse et al., 1983). The second spinal nerve also has a dorsal ramus and dorsal root ganglion. A third state of the anteriormost spinal nerve occurs in caecilians. The first spinal nerve not only is present, but is composed of dorsal and ventral roots and a dorsal root ganglion. The main branch of the ventral root contributes to the ramus hypoglossus, together with a branch of the second spinal, and in some species the occipitalis, and/or a branch of the vagus, and/or a branch of spinal 3 (Wake, 1992). Retention of the presumed ancestral state of the first spinal nerve is

unexpected, given the profound secondary modification from a limbed to a limbless predator, and the extensive association of anterior spinal nerves with the feeding mechanism.

Caecilian vs. anuran and urodelan brainstem structure

Phylogenetic history. All living members of the Gymnophiona lack limbs, girdles, and the associated musculature and innervation, including the absence of brachial and spinal plexuses. Concomitant to limb loss, the body of caecilians has become very elongate (90–285 vertebrae; Taylor, 1968), though the tail is lost, or nearly so, in all taxa. Limb loss and its correlates present a pattern of morphology distinctly different from that of salamanders, even the elongate taxa, and of frogs. No members of the Anura or the Caudata have lost all of their limbs, though they may be reduced (and hindlimbs lost) in salamanders; all retain the appropriate musculature and its innervation. Limb loss and secondary simplification of the tongue have been conjectured by Wake (1992) to provide conditions that permit morphological innovation, such as incorporation of additional nerves (occipitalis, rami of the vagus and of spinal 3) into the ramus hypoglossus.

The brainstem of salamanders and frogs is not limited by the end of the skull, but is confluent with the anterior spinal cord (summarized by Roth et al., 1990). A similar situation exists in caecilians, in which the brainstem extends well posterior to the foramen magnum. In fact, the caecilian brainstem is more elongate than that of elongate salamanders. We speculate that the combination of reduction of morphological structure and overall body elongation has permitted the elongation of the brainstem as well. We cannot explain the presence of the occipital nerve, since it may be either a retention of the ancestral condition, or a reacquisition. However, the constitution of spinal nerve 1 by dorsal and ventral roots and a dorsal root ganglion is apparently a retention of the ancestral vertebrate condition, found in all caecilians examined, but lost in salamanders and frogs. It is possible that reduction of both limb and tongue components otherwise innervated by spinal nerve 1 might "permit" retention of the ancestral condition without specialization.

Ontogenetic history. Little is known about caecilian development, especially that of the brain. The length of the developmental period is known for only a few species. However, all indications are that time to metamorphosis or birth is protracted in caecilians, for the free-living larval period is approximately one year (*Ichthyophis glutinosus*: Breckenridge and Jayasinghe, 1979; Breckenridge et al., 1987; Philippine *Ichthyophis* spp.: Taylor, 1960). The gestation period in viviparous species is also long in the only taxa for which there are data, 11 months in *Dermophis mexicanus* and *Gymnopsis multiplicata*, and 7–9 months in *Typhlonectes compressicauda* (summarized in Wake, 1993b).

Development in caecilians is highly cephalized, with head morphology much advanced over that of the posterior part of the body early in gestation (see Wake and Hanken, 1982). Comparative ontogenetic studies of amphibians suggest that heterochronic processes during development have a major influence on the degree of lamination, formation of nuclei, and number of migrated cells within the brain (Schmidt and Wake, 1991; Roth et al., 1993; Schmidt and Roth, 1993). During brain development, the lateral motor column is constituted by cells that migrate from the

periventricular ependymal layer to the peripheral neuropil. Studies of frogs (Van Mier et al., 1985) and salamanders (Nishikawa et al., 1991) show that this occurs after the medial motor column has been constituted. The absence of the lateral motor column in bolitoglossine salamanders (Wake et al., 1988) is thought to be a consequence of paedomorphosis, in that later ontogenetic events (i.e., the migration of cells into the periphery to form the lateral column) do not occur. Paedomorphosis has effected what Roth et al. (1993) call a "secondary simplification" of brain morphology. The brain of caecilians is characterized by a similar simplification. There is little or no lamination, and there are only a few migrated cells in the superficial neuropil in most brain regions (Schmidt and Wake, 1991). However, in general, caecilians have more migrated cells within the brain than do salamanders. *Typhlonectes* has neurons that form a lateral motor column. Comparative ontogenetic studies on cellular migration within the tectum mesencephali of all three amphibian orders (Schmidt and Roth, 1993; Schmidt and Wake, unpublished data) indicate that in caecilians, late ontogenetic processes are not retarded to the degree that they are in salamanders. The complexity of the brainstem in caecilians, more similar to that of frogs than to that of salamanders, may be a consequence of the early development of the medulla oblongata, since retardation largely affects later-developing brain regions.

Brainstem organization in caecilians therefore differs from that of their sister taxa, salamanders and frogs, as a consequence of (1) a combination of phylogenetic constraints, perhaps associated with limb loss and simplification of the tongue, that are especially reflected in ontogeny, and (2) particular features of neural ontogeny, such as pattern of cell migration and motor column establishment. This study presents baseline data for one species of the Order Gymnophiona, and introduces a series of questions, both technical (degree of staining of sensory pathways, staining of lateral occipital nucleus components) and/or empirical (projection patterns of neurons of distinct cytoarchitectural characteristics, anterograde staining to specify innervation patterns by particular nerve rami, comparative and ontogenetic biology). We propose to resolve these issues in the near future with a series of targeted experiments utilizing a broader array of techniques and of taxa.

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