

The Structure of the Brainstem and Cervical Spinal Cord in Lungless Salamanders (Family Plethodontidae) and Its Relation to Feeding

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ABSTRACT

We present an HRP study of the sensory tracts and motor nuclei associated with feeding (especially use of the tongue) in plethodontid salamanders (mainly *Batrachoseps attenuatus*, *Bolitoglossa subpalmata*, *Desmognathus ochrophaeus*, *Eurycea bislineata*, and *Plethodon jordani*). The nerves studied are VII (ramus hyomandibularis only), IX, X, XI, the first spinal nerve (hypoglossus), and the second spinal nerve. Two types of sensory projections are universally found in the brainstem: superficial somatosensory projections of VII, IX, and X, and deeper visceral sensory projections of IX and X to the fasciculus solitarius. The first spinal nerve and the spinal accessory nerve (XI) have no sensory projections, but the second spinal nerve has typical projections along the dorsal funiculus of the spinal cord.

The motor nuclei of VII ramus hyomandibularis, IX, and X form a combined nucleus situated at the level of the IX/X root complex. The nucleus of the first spinal nerve is well separated from the combined nucleus and is situated rostral and caudal to the obex. The rostral part of the motor nucleus of the second spinal modestly overlaps that of the first. The motor nucleus of the spinal accessory nerve is more or less restricted to the region of the second spinal nerve. Its fibers leave the brain through the last root of the IX/X complex and the related ganglion. Bolitoglossine and nonbolitoglossine differ in the architecture of the spinal nuclei. Two distinct types of motor neurons occur in spinal nuclei of nonbolitoglossine species—some of those with tongue projection—but only one type is found among the tongue-projecting bolitoglossine group.

Key words: motor nuclei, sensory pathways, tongue, urodeles

The feeding system of plethodontid salamanders has been the subject of study in our laboratories for many years (Lombard and Wake, '76, '77; Rettig and Roth, '82; Roth, '76, '78; Roth et al., '83, '84; Thexton et al., '77; Wake, '66, '82; Wake and Lombard, '71, '73; Wake et al., '83). Special attention has been given to the species with tongue projection abilities. The projectile tongue of plethodontids contains a skeleton and several muscles. This complex structure differs from that of anurans in fundamental ways in the mechanisms of projection.

All members of the family Plethodontidae are lungless, and this circumstance has been of great significance for the evolution of tongue projection ability. The projectile is built

from the hyobranchial skeleton and associated tissues (Fig. 1). These structures are used to fill the lungs of other urodeles, but in plethodontids the release from this functional constraint has permitted the molding of this complex of cartilages, ligaments, muscles, nerves, and other tissue into a highly specialized adaptation for catching prey (Wake, '82; Roth and Wake, '85). Our earlier report on patterns of peripheral innervation of the muscles involved in tongue projection (Wake et al., '83), together with the more general accounts of comparative morphologists and neurologists

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(e.g., Drüner, '04; Francis, '34; Herrick, '48), provides a foundation for this work. In this paper we extend our work to the brainstem and cervical spinal cord, which have not been studied previously in urodeles with modern techniques.

A pair of muscles (subarcualis rectus I—SARI) extends from the ceratohyals to the epibranchials. When activated, the articulated hyobranchial skeleton (a basibranchial, two pairs of ceratobranchials, and a pair of epibranchials) and a tongue pad attached to its anterior end are moved rapidly forward out of the mouth. This apparatus is directed on its outward course by a firing cylinder involving the suprapericardialis (the "barrel") and the geniohyoideus lateralis muscle as important components. As the apparatus leaves the mouth it carries with it the muscles which restrain its forward movement and return it to the mouth. The geniohyoideus muscles extend from the lower jaw into the tongue pad and ordinarily greatly restrict tongue projection. They are absent in the species with greatest tongue projection abilities. The apparatus is returned to the mouth through the action of the rectus cervicis-rectus abdominis complex. The tongue pad has several muscles that act to mold the tongue in various ways during projection. All of these muscles are controlled by the last few cranial nerves and the cervical nerves.

The SARI is a branchiomeric muscle and the remainder are members of the hypobranchial series. Accordingly, we have sought to understand the integration of the elements at a neuroanatomical level. The most specialized tongue projection system of plethodontids is the fastest feeding reaction known among vertebrates (Theuton et al., '77). The muscles involved in the feeding reaction contract more or less synchronously. The animal experiences a convulsion-like phenomenon in its head and neck which results in the tongue being projected and instantly retracted. This is the result of the geometry of the peripheral structure (e.g., the differing length-tension curves of the muscles and the shape

and attachment of the cartilage, muscles, and connective tissues).

In the present paper our attention is focused on the central projections of primary sensory fibers and on the cells of origin of motor fibers of the nerves innervating the muscles related to the feeding system. These are the branchiomeric nerves (cranial nerves V, VII, IX–XI) which emanate from the medulla oblongata, and the first (homologue of cranial nerve XII) and second spinal nerve. We disregard cranial nerve V, which is responsible for jaw closure but does not relate to the hyobranchial apparatus, and we consider only some especially relevant parts of VII and X.

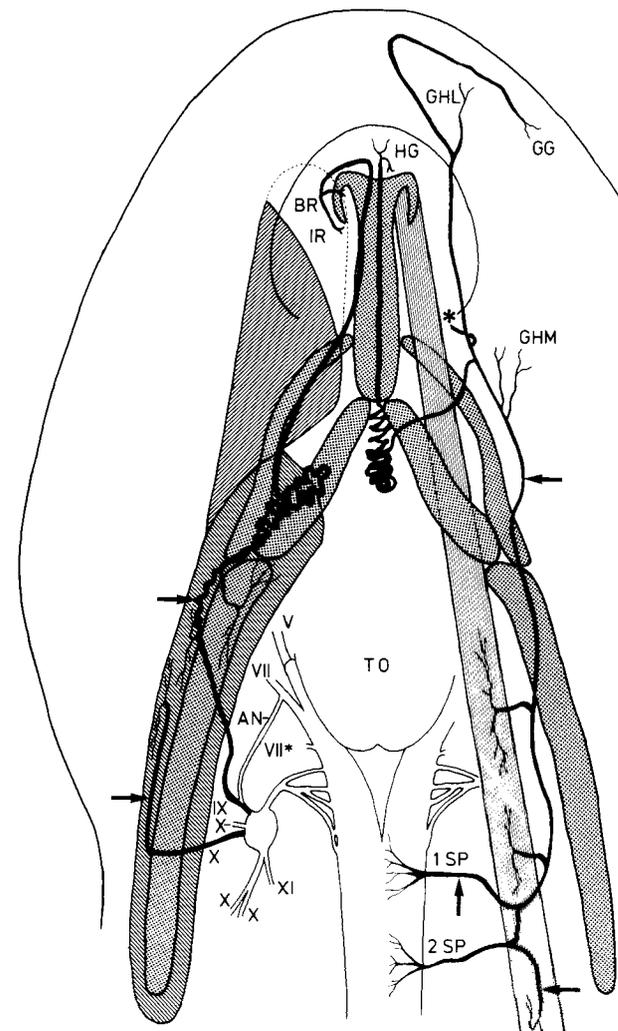


Fig. 1. Pattern of innervation of the tongue and associated musculature of a bolitoglossine plethodontid, here *Batrachoseps attenuatus*. The figure is composed of ventral and dorsal views of the hyobranchial apparatus and the brain. It shows the lower jaw with the tongue skeleton (stippled area), the tongue protractor muscle *m. subarcualis rectus I* (SARI, left side, hatched area), the tongue retractor muscle *m. rectus cervicis profundus* (RCP, right side, parallel-lined band), the course of the nerves supplying SARI (left side) and RCP (right side), and the outlines of the tongue pad and the brainstem and cervical spinal cord. Arrows indicate sites of nerve sectioning and labeling with HRP. For further explanation see text.

Abbreviations

AN	Anastomosis between nerves VII and IX
BR	M. basibranchialis
GG	M. genioglossus
GHL	M. geniohyoideus lateralis
GHM	M. geniohyoideus medialis
G IX/X	Ganglion of nerves IX and X
HG	M. hyoglossus
IR	M. interradians
MN VII, IX, X	Combined motor nucleus of nerves VII (r. hyomandibularis), IX and X
MN X*	Motor nucleus of the entire nerve X
MN XI	Motor nucleus of nerve XI
MN 1SP	Motor nucleus of first spinal nerve
MN 2SP	Motor nucleus of second spinal nerve
NCI	Neuropil of nerve X at the commissura infima
N VII	Nervus facialis
N VII RH	Nervus facialis, ramus hyomandibularis
N IX	Nervus glossopharyngeus
N X	Nervus vagus
N XSAR	Nervus vagus, branch innervating <i>m. subarcualis rectus</i>
N XI	Nervus accessorius spinalis
RCP	M. rectus cervicis profundus
SARI	M. subarcualis rectus I
TO	Tectum opticum
1SP	First spinal nerve
2SP	Second spinal nerve
2SPD	Dorsal root of second spinal nerve
2SPV	Ventral root of second spinal nerve

MATERIALS AND METHODS

We chose species belonging to genera that display a variety of life histories and tongue projection abilities. Life histories are important because those species that have an aquatic larval stage appear to have been constrained to particular pathways in evolution (Lombard and Wake, '77; Wake, '82; Roth and Wake, '85). Genera with aquatic larvae include *Desmognathus* (*D. monticola*, *D. ochrophaeus*), which has a stout pair of genioglossus muscles and little ability to project the tongue, and *Eurycea* (*E. bislineata*, *E. junaluska*), which has lost the genioglossus muscle and has a projectile tongue. The other genera studied all lack an aquatic larval stage and have direct development from eggs on land. *Plethodon* (*P. cinereus*, *P. jordani*) has stout genioglossus muscles and relatively restricted tongue-projecting ability. *Batrachoseps* (*B. attenuatus*) has genioglossus muscles that are greatly elongated, and the genus has good tongue-projecting ability. *Bolitoglossa* (*B. subpalmata*) lacks genioglossus muscles and has an exceedingly fast and highly projectile tongue. *Hydromantes* (*H. italicus*) also lacks genioglossus muscles, and while its tongue is somewhat slower than that of *Bolitoglossa* (Roth, '76), it has the most projectile tongue of all urodeles. We have also examined members of the genera *Thorius* (*T. narisovalis*), *Oedipina* (*O. uniformis*), and *Nototriton* (*N. picadoi*), which all have a tongue rather like *Bolitoglossa*.

The general anatomy of muscles and nerves related to feeding is shown in Figure 1. Several members of each genus were selected for careful dissection, following anaesthetization in MS 222 (concentration 1:100). These specimens were used to determine the main pattern of peripheral innervation, and these were compared to serial sections stained by the Palmgren method which were available for several specimens of each genus. The primary technique involved utilization of horseradish peroxidase (HRP) to fill individual nerve cells and their projections, which were studied in frozen sections and whole mounts cleared in methyl salicylate once they had been stained. In total, about 140 brains were studied by means of the HRP method.

Our HRP method was as follows: horseradish peroxidase (Boehringer, grade I) was applied in crystals to the cut stump of the nerves both peripherally and near the entrance to the brain. The sites of labeling were the following: the ramus hyomandibularis of nerve VII was sectioned near the mandibular quadrate-articular joint; nerves IX and X were sectioned immediately before entering the SAR muscle (nerve IX before the separation of motor fibers innervating SAR and the ramus lingualis); first spinal nerve in the gular region (ramus hypoglossus) and between the brain and the commissure between the first and second spinal nerves; second spinal nerve before entering the RCP muscle; spinal accessory nerve after leaving the IX/X ganglion.

After a survival time of 48–72 hours the animals were perfused with isotonic saline, followed by a solution of 1% glutaraldehyde + 1% paraformaldehyde + 2% sucrose in 0.12 M phosphate buffer (pH 7.4). The brains were rinsed in 0.1 M cacodylate buffer (pH 7.2) for 2 hours. They were treated as whole mounts in a solution of 0.2% diaminobenzidine (Sigma) + 0.01% H₂O₂ in 0.1 M cacodylate buffer (pH 5.45) for 1 hour. They were cleared in methyl salicylate (oil of wintergreen) and photographed as whole mounts. In order to make serial reconstructions of the motor nuclei

and neurons, whole mounts of *Batrachoseps* and *Plethodon* were returned to alcohol and after standard procedure embedded in Epon and cut in transverse sections of 30 μ m.

RESULTS

General description of nerves involved in the control of feeding

Four nerves are involved in the control of tongue projections: cranial nerves IX (glossopharyngeus) and X (vagus), and the first (= cranial nerve XII, hypoglossus) and second spinal nerves. Additionally, ramus (r.) hyomandibularis of cranial nerve VII (facialis) controls the opening of the mouth and the main ventral throat constrictors, and cranial nerve XI (accessorius spinalis) controls head movement.

The fibers of the r. hyomandibularis leave the brain either through the main root of VII or through a separate root (VII* of Fig. 1) and innervate the ventral main m. interhyoideus, the lateral and ventral m. interhyoideus posterior, and the lateral and dorsal m. depressor mandibulae. Cranial nerves IX, X, and XI have a common ganglion. Between the ganglion and the hindbrain four roots exist (Figs. 1, 7). The first contains sensory and motor fibers and belongs exclusively to nerve IX. The second is sensory and contains fibers of IX and X, and additionally of r. hyomandibularis of VII that enter the ganglion through the anastomosis between VII and IX. The small third root contains motor fibers of X. The last root of this complex is strictly motor and belongs to nerve XI (Roth et al., '84).

The glossopharyngeus leaves the ganglion most rostrally. It innervates the m. subarcualis rectus I (SARI), the tongue protractor muscle. A major sensory branch, the ramus lingualis, proceeds anteromedially toward the tongue pad where it supplies the m. basiradialis and m. interradians (Fig. 1). The second nerve leaving the ganglion has vagal functions and is not related to the tongue. It lies in close proximity to a small nerve that represents the vagal innervation of the SARI. A fourth large nerve divides into two large branches which are primarily involved in parasympathetic pathways. A final nerve, the accessorius spinalis, extends caudally paralleling the spinal cord. It sometimes leaves the ganglion with the fourth nerve before separating from it (Fig. 7). The accessorius spinalis is exclusively motor and innervates the m. levator scapulae and the m. cucularis. A medium-sized sensory nerve emerges from the dorsal part of the ganglion and soon separates into several branches to serve the lateral neck region.

The first spinal nerve (or hypoglossus) has no dorsal ganglion in adult salamanders. It is formed from several ventral rootlets. It gives off a branch to the neck musculature and then extends cephalad along the ventral musculature as the ramus hypoglossus (Fig. 1). The first spinal nerve is strictly motor and serves the musculi (mm.) genioglossus, geniohyoideus medialis, geniohyoideus lateralis, supradyndicularis, and muscles of the tongue pad, especially the m. hyoglossus.

The second spinal nerve has both dorsal and ventral roots and a ganglion. It gives off a branch to the neck and then it divides into two rami. The more posterior extends toward the arm where it joins the brachial plexus. The more anterior innervates the mm. rectus cervicis superficialis and omohyoideus. This ramus gives off a small nerve which a commissure between the two nerves. Just before and immediately after joining the first spinal it innervates the m. hebesteoypsiloideus, and another one, or two, coiled

branches serve the m. rectus cervicis profundus (RCP), the main tongue retractor muscle (Fig. 1).

Sensory tracts of the brainstem and cervical spinal cord

R. hyomandibularis of the n. facialis. There are two distinct pathways of the hyomandibular branch of the facial nerve to the brain. One is via the main ramus dorsally through the facial ganglion to the brain (Fig. 6A, pathways

1 and 2). The other follows the hyomandibularis until the ventral surface of the otic capsule is reached. It then enters a large commissure that extends posterodorsally to join the main ramus of the glossopharyngeus a short distance before entering the IX/X ganglion (Figs. 1, 2A; Fig. 6A, pathways 3 and 4). This ganglion also contains numerous cell bodies of the ramus hyomandibularis.

Behind the facial ganglion the first sensory pathway separates into two distinct sensory projections, both joining the

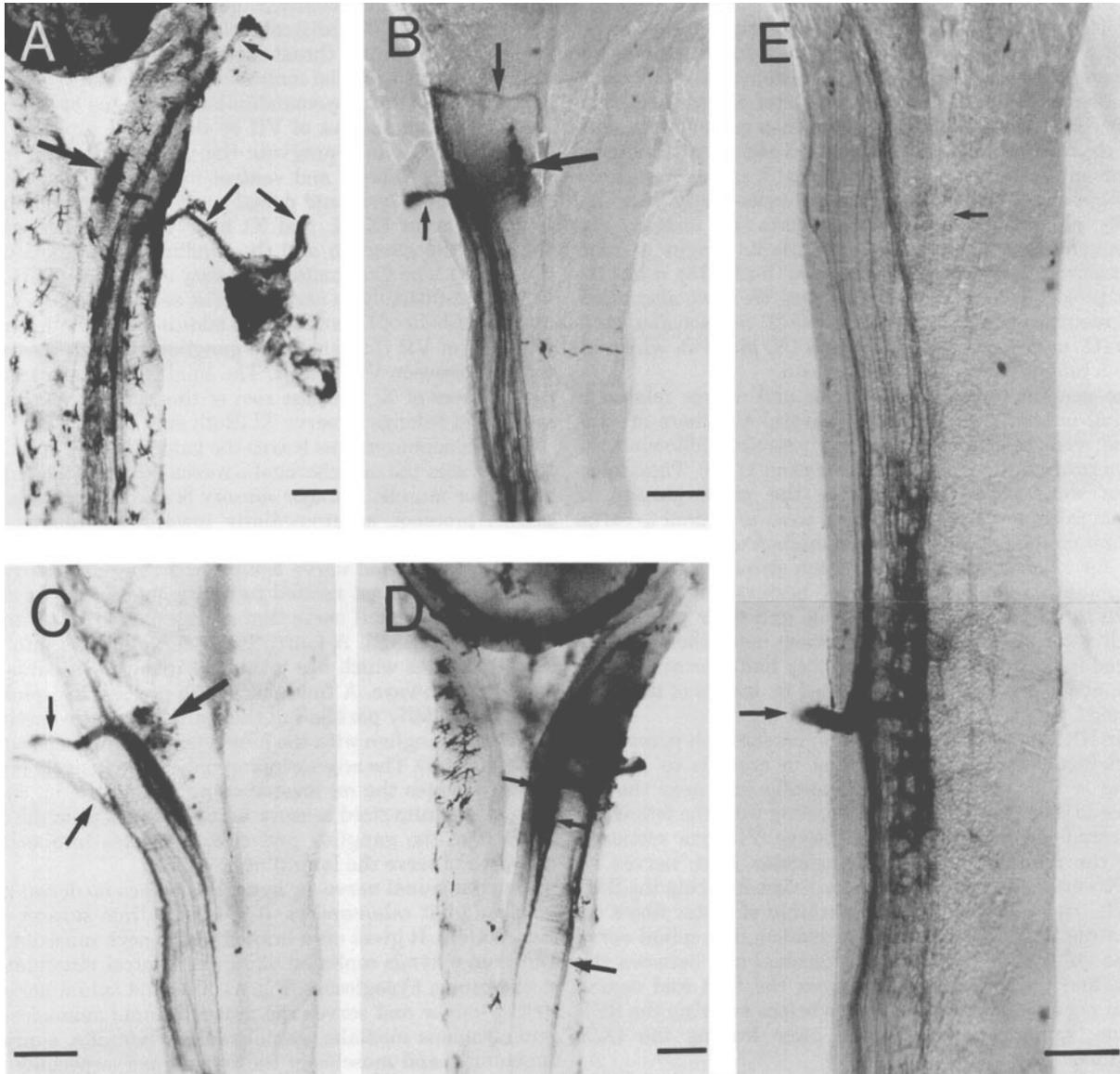


Fig. 2. Sensory and motor pathways of the brainstem and cervical spinal cord related to feeding. A. Projections of ramus hyomandibularis of n. facialis (VII). Whole mount of the brain of *Plethodon jordani*, dorsal view. Thin arrow, pathway through the main root of VII; medium arrows, pathway through the VII-IX anastomosis and the second root of the IX/X/XI root complex; thick arrow, motor nucleus of r. hyomandibularis. B. Sensory and motor pathways of r. hyomandibularis in *Desmognathus ochrophaeus*. Whole mount, dorsal view of the brain. Thin arrow, sensory pathway through IX/X/XI root complex; medium arrow, motor pathway; thick arrow, motor nucleus of r. hyomandibularis. C. Sensory and motor pathways of n. glossopharyngeus (IX). Wholemount of the brain of *Batrachoseps attenuatus*, dor-

sal view. Thin arrow, sensory pathway through the first root of the IX/X/XI root complex, entering the fasciculus solitarius; medium arrow, sensory pathway through the second root, entering the superficial somatosensory fiber band of the tractus descendens V; thick arrow, motor nucleus of IX. D. Sensory pathways of IX. Wholemount of the brain of *Plethodon jordani*, dorsal view. Thin arrows, medial and lateral bundles of the fasciculus solitarius; medium arrow, somatosensory fibers of the tractus descendens V. E. Sensory projections of the second spinal nerve. Wholemount of the brain of *Bolitoglossa subpalmata*, dorsal view. Thin arrow, obex region; medium arrow, dorsal root. Calibration bar = 200 μ m.

tractus descendens tractus descendens V. The more anterior one enters the brain through the dorsal part of the facial root. This branch runs dorsomedially and then descends superficially and medially almost to the obex (Fig. 6A, pathway 1). The more posterior projection leaves the ganglion along the anteroventral margin of the root of the facialis, but it moves posteroventrally and passes below that root to have its own entry to the brainstem (Fig. 6A, pathway 2; Fig. 2A). This projection, at first rather ventral, gradually moves dorsally as it descends below the first root of the IX/X complex to join the descending superficial sensory pathways of the Xth nerve. At this level the projection is broad and equivalent in size to the projection joining it. The joint projection extends far down the spinal cord at least to the level of the fourth spinal nerve.

The second pathway of the ramus hyomandibularis, following the commissure between the facial and glossopharyngeal nerves and passing through the IX/X ganglion, enters the brainstem through the second of the roots leaving the ganglion (Fig. 2A,B). The fibers form two superficial ascending and descending tracts, one medial (Fig. 6A, pathway 3) and the other more lateral (Fig. 6A, pathway 4). The latter tract merges with the posterior sensory projection of the first pathway of the ramus hyomandibularis described above and descends far posteriorly. At the level of the second spinal nerve the medial and lateral tracts are joined by a dense riblike meshwork of fibers. From this meshwork fibers penetrate the gray matter, and a number proceed contralaterally.

N. glossopharyngeus. Sensory fibers of the glossopharyngeus enter the brain through the first two roots of the IX/X/XI root complex (Figs. 2C, Fig. 6A). Fibers of the first root from the ganglion run dorsally toward the ridge of the rhombencephalon and join the fasciculus solitarius formed by viscerosensory fibers of nerves V, VII (exclusive of r. hyomandibularis, as described above), IX, and X. The fasciculus solitarius runs along the medial margin of the ridge in a dorsal position, somewhat below the surface (in *Batrachoseps* about 30 μm deep), and is half-surrounded by the cells of the nucleus fasciculi solitarii. Some fibers leave the fascicle, mainly in a mediodorsal direction into the nucleus fasciculi solitarii and in a ventral direction toward the dendrites of the VII, IX, and X motor neurons.

The glossopharyngeal portion of the fasciculus solitarius consists of two closely applied bundles, one more medial and the other more lateral (Figs. 2D, Fig. 6A). The more medial bundle contains mostly descending fibers that form a rather dense neuropil that extends as far as the obex. The more lateral one has both ascending and descending fibers. The ascending tract, which has fewer fibers than the descending one, extends along the rhombencephalic ridge to the most rostral part of the hindbrain, where it turns medially and disperses into fine twigs in the vicinity of the nucleus visceralis secundarius. The descending tract becomes superficial at the level of the obex and extends along the midline caudally at least to the level of the third spinal nerve.

Most of the fibers entering the brain through the second root do not join the fasciculus solitarius but form a broad superficial dorsolateral tract (in *Batrachoseps* about 30 μm thick) (Figs. 2C,D, Fig. 6A). The fibers ascend and descend, joining the tractus descendens nervi trigemini. The ascending fibers terminate at the same level as those of the fasciculus solitarius, while the descending fibers form a very broad tract that gradually moves to a more dorsal and

medial, but still superficial position. This broad tract gradually thins out, descends further in Lissauer's tract, and disappears far caudally near the level of the fourth spinal nerve. A few fibers move dorsally to join the fasciculus solitarius while others constitute a narrow and ill-defined tract between the fasciculus solitarius and the superficial tract. Posteriorly to the obex fibers of the sensory tract of IX intermingle with fibers of the dorsal root of the second spinal nerve. Large numbers of interchanging fibers move between the dorsomedial and dorsolateral tracts.

N. vagus. Sensory fibers of the vagus enter the brain through the second root from the ganglion, together with fibers from the r. hyomandibularis and the glossopharyngeus, and join the tractus descendens. Only a few fibers move rostrally, but large numbers proceed caudally in Lissauer's tract. The vagus contributes only a few fibers to the fasciculus solitarius. A second vagus projection, from the lateral neck musculature, contributes fibers via the second root from the ganglion. These ascending and descending fibers join both the medial and lateral portions of the fasciculus solitarius. Just rostral to the obex a medial tract diverges from the medial portion and moves toward the obex, where it forms a dense neuropil in the commissura infima, from which ascending and descending fibers project a short distance contralaterally. The ipsilateral fibers extend far caudally, at least to the level of the fourth spinal nerve (Fig. 6B).

N. lateralis posterior. A final sensory projection associated with the IX/X ganglion enters the brain from a posterior position paralleling the main ramus of the accessorius spinalis. Its entrance to the brain is together with the first root of the IX/X complex. This projection ascends and descends in paired parallel bundles which lie superficial to the fasciculus solitarius. The ascending bundles terminate in the auricles at the level of the lateral origin of the cerebellum. The descending bundles terminate just before reaching the obex. This sensory projection was found only in aquatic and semiaquatic species of *Eurycea* and *Desmognathus* and belongs to the lateral line system of these species (Fig. 4).

Second spinal nerve. There is an extensive sensory projection through the dorsal root of the second spinal nerve. This root is located about halfway between the ventral roots of the second and third spinal nerves (Fig. 2E). Broad ascending and descending projections run along the dorsal funiculus as the apparent homologue of Lissauer's tract. Fibers pass between the two projection bands in a broad region on either side of the root. The ascending fibers meet descending ones from the second root of the IX/X complex and continue forward past the obex with some fibers reaching the rostral pole of the hindbrain. Many fibers of the tracts terminate deep within the dorsal and dorsolateral periventricular gray matter. Some fibers cross the midline and project to the contralateral side.

Finally, we found no sensory projections in either the spinal accessory or first spinal nerves. No differences in sensory projections of larvae and adults were detected in the species examined (*Eurycea bislineata*, *E. junaluska*).

Distribution of motor tracts and nuclei

The motor nucleus of hyomandibularis, glossopharyngeus, and vagus. The nuclei of the entire nervus glossopharyngeus, the ramus hyomandibularis of the nervus facialis, and that portion of the nervus vagus serving the SARI are intermingled to form a continuous column. This

combined nucleus is situated in the ventrolateral portion of the gray matter, below the sulcus limitans and medial to the fasciculus solitarius (Fig. 2A–C). It extends from the level of the first root of the IX/X complex to the level of the third root. In all plethodontid species studied as far it has an elongate oval shape. The first two motor tracts exiting this nucleus belong to ramus hyomandibularis of the nervus facialis. These motor fibers leave the medial and caudal parts of the nucleus medially, then extend rostrally near the midline in several small fiber tracts. Near the point of exit they turn sharply in a lateral direction and leave the brain either together with the more posterior of the sensory roots of the r. hyomandibularis or as a separate motor root medial and caudal to it (Fig. 2B).

The second component of this nucleus is the motor portion of the nervus glossopharyngeus, which leaves the brain dorsally together with sensory fibers as the first root of the IX/X complex. The fibers pass directly from the nucleus in a dorsolateral direction to their point of exit (Fig. 2C). The final component of this nucleus is the vagus innervation of the SARI and its fibers exit the brain via the third root of the IX/X complex. The fibers originate in the posterior part of the nucleus. The motor component of the entire vagus complex is relatively large and elongate. The portion serving the SARI is located at its anterior end. The whole vagus nucleus extends posteriorly approximately to the level of the obex.

In *Batrachoseps attenuatus* the combined motor nucleus of VII, IX, and X related to feeding is 275 μm long and 112 μm wide (Fig. 7A). It contains about 70 neurons, about half of which belong to the glossopharyngeus motor nerve. The diameter of these cells ranges from 13 to 18.7 μm with a mean of 15.7 μm . The cells are all situated at the ventrolateral border of the periventricular gray matter. The dendritic trees are rather narrow. All neurons of the anteromedial (glossopharyngeus/hyomandibularis) part of the nucleus and most of the posterior (hyomandibularis/vagus) extend their dendrites in a ventrolateral direction toward the pial surface forming a very dense neuropil. In the posterior part some medially situated neurons project their dendritic trees ventromedially (Fig. 5A). There are, however, no dendrites crossing the midline to the contralateral side.

The length of the VII/IX/X nucleus in *Bolitoglossa* is 280 μm (Fig. 7B), which is almost the same as in *Batrachoseps*. In *Plethodon* the nucleus has a length of 340 μm (Fig. 7C). Shape and arrangement of the neurons are the same as in *Batrachoseps*.

The motor nucleus of first spinal or hypoglossal nerve. The size of this nucleus varies considerably among species of plethodontids. In *Batrachoseps attenuatus* it is 687 μm long and 75 μm wide. It extends from the last quarter of the rhombencephalon to the midpoint between the ventral roots of the first and second spinal nerves (Fig. 7A). In *Bolitoglossa* it is 1,280 μm long and 140 μm wide and extends more caudad than in *Batrachoseps*, almost to the entrance of the ventral root of the second spinal nerve (Fig. 7B). In *Plethodon jordani*, the nucleus is shorter (980 μm) than in *Bolitoglossa*, but much wider (200 μm) (Fig. 7C). This corresponds with the fact that in *Batrachoseps* and *Bolitoglossa* the motor neurons are of similar size and shape and are arranged rather irregularly in an elongate cluster, whereas in *Plethodon* there are two parallel rows of cells of different size.

The motor nucleus of *Batrachoseps attenuatus* consists of about 60 neurons with diameters between 13 and 17.3 μm with a mean of 15.2 μm (Fig. 3A). They are situated at the periphery of the periventricular gray matter from a ventromedial to a ventrolateral position. The neurons are always pear-shaped. Most have rather narrow dendritic trees which extend to the pial surface in a more or less lateral or horizontal direction. Only a few dendritic branches extend in a ventral or dorsal direction (Fig. 5B).

In *Plethodon jordani* the motor nucleus consists of two rows of cells, a medial one containing pear-shaped neurons with a mean diameter of 15.5 μm and dendritic trees extending from ventromedial to lateral, and a lateral one of large spindlelike neurons with a mean major axis of 24.6 μm (range 19–28.5 μm) and very wide dendritic arborization extending from ventromedial to dorsolateral. Some dendrites extend far into the contralateral side. Directly on the midline of the medulla oblongata bipolar cells are found that extend their dendritic trees into both halves, mostly in a lateral and dorsolateral direction (Fig. 5E).

Motor nucleus of the second spinal nerve. The motor root of the second spinal nerve includes diverse components from the neck, shoulder, and arm musculature (small contributions to the brachial plexus). In *Plethodon* some fibers proceed to the tongue via an anastomosis joining the first spinal nerve (Fig. 1). In *Batrachoseps* and *Bolitoglossa* fibers from the second spinal proceed via the anastomosis, but shortly after joining the second spinal nerve these fibers leave to innervate the rectus cervicis muscles (Roth et al., '84).

The motor nucleus of the second spinal again varies in size and architecture among plethodontid species. In *Batrachoseps attenuatus* it is 812 μm long and 127 μm wide in its anterior part and 62 μm wide in its posterior part (Figs. 3B, 7A). It thus is longer and partly wider than the hypoglossal nucleus. The second spinal nucleus extends from the level of the last rootlets of the hypoglossal nucleus to the midpoint between the ventral roots of the second and third spinal nerve. There is an overlap of 150 μm in length between the two motor nuclei. In *Bolitoglossa subpalmata* the nucleus of the second spinal is shorter than the hypoglossal nucleus, its length being 860 μm . There is an overlap of 340 μm between the two nuclei. The width is 160 μm (Fig. 7B). In both species the motor neurons are arranged rather irregularly in a ventromedial to a ventrolateral position at the margin of the periventricular gray matter. These neurons are more densely packed than are those of the hypoglossal nucleus.

In *Batrachoseps* the nucleus contains 40–50 neurons. Their diameters range from 14.4 to 23 μm with a mean of 17.3 μm . Relative to the previous motor neurons those of the second spinal show a much wider dendritic arborization extending from a ventrolateral to a dorsolateral position (Fig. 5C). Some dendrites follow the surface of the periventricular gray matter in a dorsal and dorsomedial direction, but do not reach the pial surface. A number of dendrites, mostly from medially situated neurons, cross the midline and extend into the ventral part of the contralateral spinal cord. All perikarya are rounded or pear-shaped, not spindle-like. In all bolitoglossines studied so far (*Batrachoseps*, *Bolitoglossa*, *Hydromantes*, *Oedipina*, *Nototriton*, and *Thorius*) there is no clear distinction in perikaryon size and shape or arborization between medially and laterally situated neurons.

In *Plethodon jordani* the motor nucleus of the second spinal has about the same length (860 μm) as in *Bolitoglossa*, but is much wider (240 μm) Fig. 3C). Here, the motor neurons are arranged in two longitudinal clusters, similar to the situation found in the hypoglossal nucleus of this species. The medial row consists of smaller neurons, mostly with pear-shaped perikarya with a diameter of 16 μm , sim-

ilar to those described above for bolitoglossines. The lateral row contains large neurons with spindlelike perikarya with a mean major axis of 31.3 μm (range 20–63 μm). The dendritic arborization of these latter neurons is very large, extending from a ventromedial to a dorsolateral direction, with the majority projecting in a lateral and dorsolateral direction. Some of the dendrites of the large neurons ter-

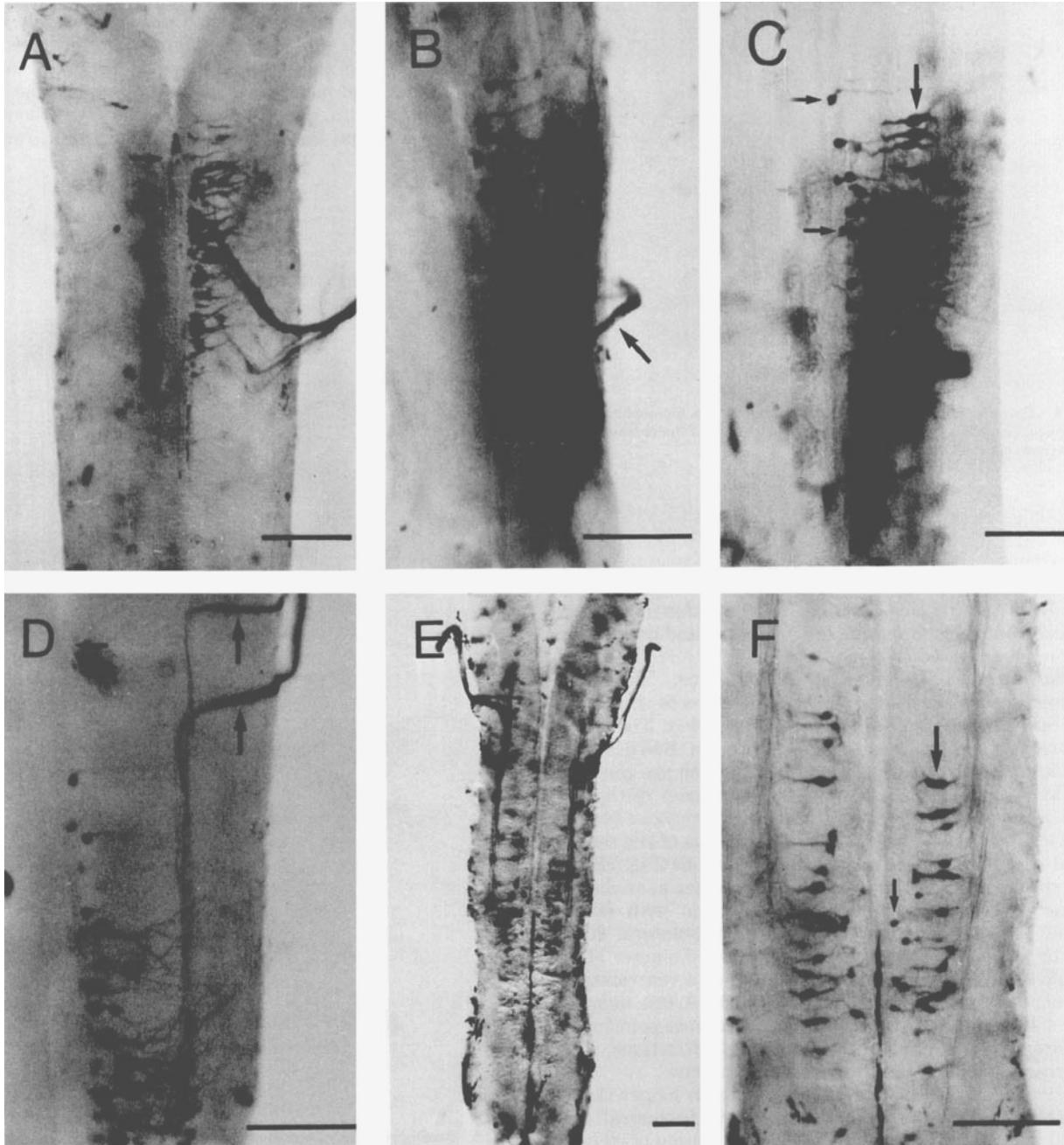


Fig. 3. Motor nuclei related to feeding. Wholemounts. A. Motor nucleus of the hypoglossus/first spinal nerve of *Batrachoseps attenuatus*. Arrow, obex region. B. Motor nucleus of the second spinal nerve of *Batrachoseps attenuatus*. Arrow, ventral root. C. Motor nucleus of the second spinal nerve in *Plethodon jordani*. Thin arrows, small medial motor neurons; medium arrows, large lateral motor neurons. D. Motor nucleus of n. accessorius

spinalis (XI) of *Bolitoglossa subpalmata*. Thin arrow, obex region; medium arrows, motor fibers of XI. E. Bilaterally stained motor nucleus of n. accessorius spinalis of *Plethodon jordani*. F. Same as E, closer view. Thin arrow, small medial motor neuron; medium arrow, large lateral motor neuron. Calibration bar = 200 μm .



Fig. 4. Posterior lateral line system associated with n. glossopharyngeus in *Eurycea junaluska*. Wholemount, dorsolateral view of the rhombencephalon. Arrow, obex. Calibration bar = 200 μ m.

minate within the sensory tracts of the second spinal nerve (Fig. 5F). This situation described for *Plethodon* (neurons of two sizes) was also found in *Desmognathus* and both larval and metamorphosed *Eurycea*.

Motor nucleus of the spinal accessory nerve. The spinal accessory nerve consists of visceral motor roots that leave the brainstem laterally at the level of the obex. The motor nucleus is variable in size and extent. In *Batrachoseps attenuatus* it consists of about 30 cells, which are confined in a loose arrangement within the boundaries of the nucleus of the second spinal (Fig. 7A). In *Bolitoglossa subpalmata* it is longer (1,100 μ m) than the nucleus of the second spinal both in anterior and posterior directions (Fig. 7B). In both species most of the neurons show either pear-shaped perikarya with a mean diameter of 17 μ m with rather narrow dendritic trees extending in a ventrolateral direction, or polygonal perikarya with a mean diameter of 23 μ m and large dendritic trees extending from a ventrolateral to a dorsolateral direction (Figs. 3D, 5D). A few neurons show spindlelike perikarya with dendritic trees pointing in opposite, ventromedial and dorsolateral, directions. Few dendritic branches cross the horizontal midline.

In *Plethodon jordani* the nucleus is again longer (1,220 μ m) than that of the second spinal both in rostral and caudal directions (Figs. 3E, 7C). The distribution of its cells is similar to that of the cells of the second spinal nucleus: in addition to smaller and medially located pear-shaped cells, large spindlelike neurons with a major axis of 29.8 μ m are found here laterally (Figs. 3F, 5G). The spinal accessory nerve, together with its motor nucleus, is present in larval and adult *Eurycea*, and in *Desmognathus*.

DISCUSSION

There are four major groups of plethodontid salamanders and they have varying degrees of specialization for feeding with their tongues (Lombard and Wake, '77). All use their tongues during terrestrial feeding, but the different groups vary in the degree to which they project their tongue. Salamanders of the subfamily Desmognathinae (represented by *Desmognathus* in the present study) have aquatic larvae and rather generalized tongues as adults. Adult tongues are attached at front and projected only moderately. All other plethodontids are in the subfamily Plethodontinae. All species of the tribe Hemidactyliini (*Eurycea*) have aquatic larvae, and most have projectile tongues that are free at the front in the adult stage. The Plethodontini (*Plethodon*) have no larval stage, but they retain rather

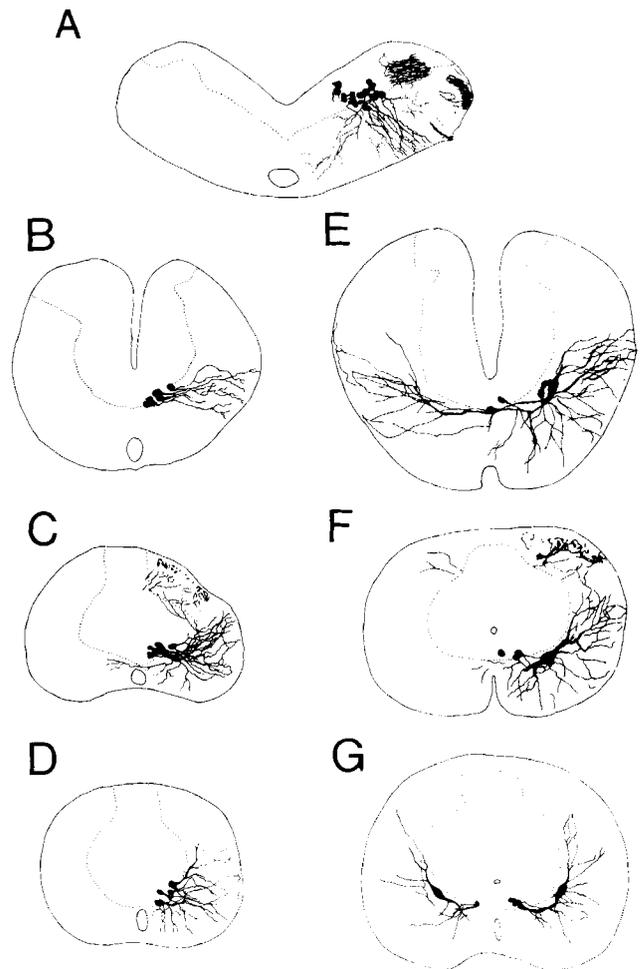
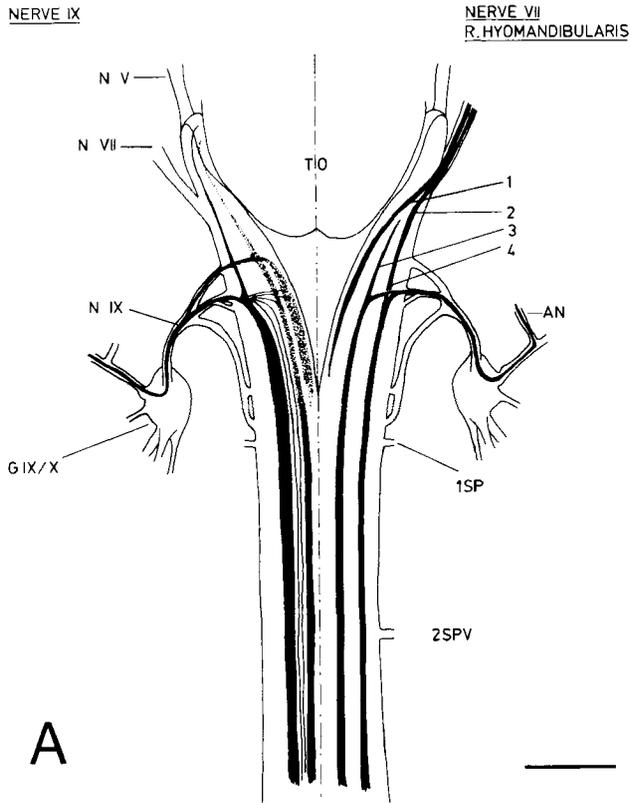
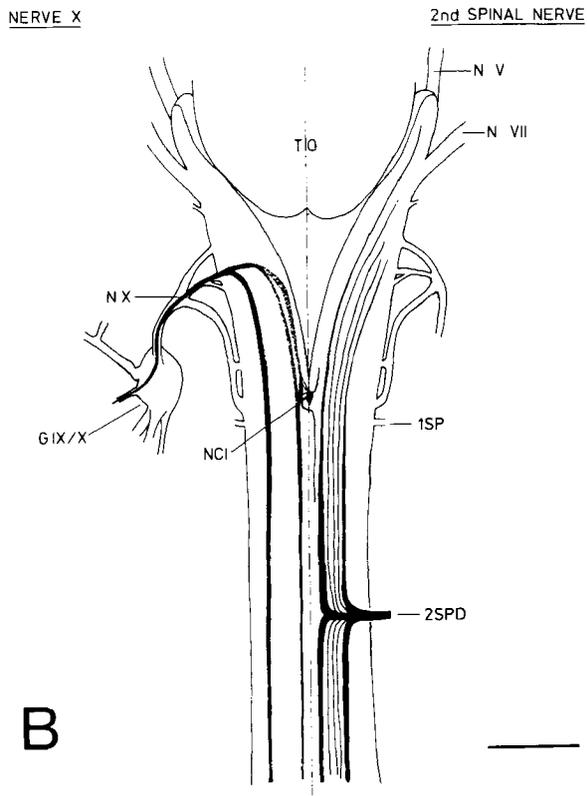


Fig. 5. Reconstructions of motor neurons related to feeding. Transverse sections through the respective nuclei. A. Nucleus VII r.h./IX/X in *Batrachoseps attenuatus*. The fasciculus solitarius is shown dorsally. B. Nucleus of first spinal in *Batrachoseps attenuatus*. C. Nucleus of second spinal in *Batrachoseps attenuatus*. The sensory tract is shown dorsally. D. Nucleus of accessorius spinalis in *Batrachoseps attenuatus*. E. Nucleus of first spinal in *Plethodon jordani*. F. Nucleus of second spinal in *Plethodon jordani*. Crossed and uncrossed fibers of the sensory tract are shown dorsally. G. Nucleus of accessorius spinalis in *Plethodon jordani*.



A



B

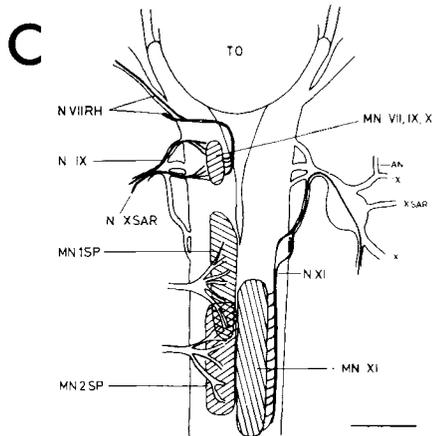
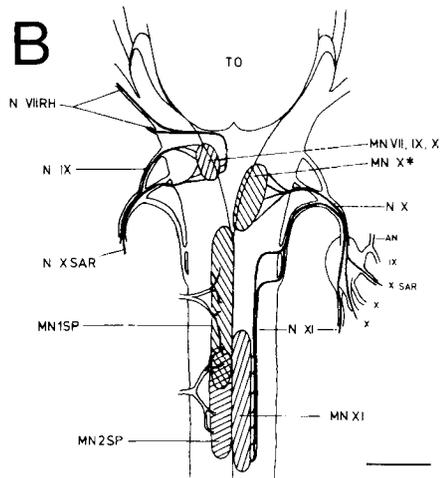
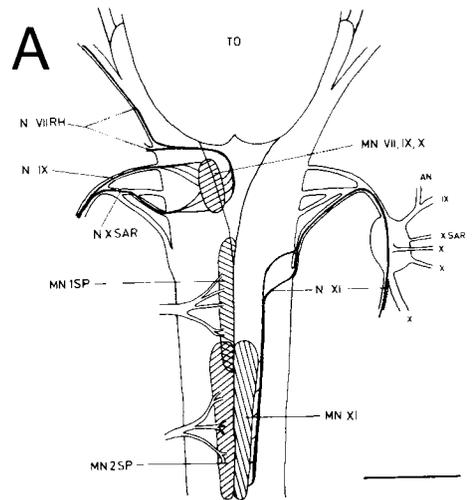


Fig. 6. Schematic drawings of dorsal sensory projections of nerves related to feeding. A. Nerve IX (left) and nerve VII ramus hyomandibularis (right). B. Nerve X (left) and second spinal (right). Stippled bands, fasciculus solitarius; solid bands, superficial tracts. Numbers in A indicate different sensory pathways of r. hyomandibularis. Calibration bar = 500 μ m.

Fig. 7. Schematic drawing of the distribution of motor nuclei and fiber tracts related to feeding. A. *Batrachoseps attenuatus*. B. *Bolitoglossa subpalmata*. C. *Plethodon jordani*. Calibration bar = 500 μ m.

general tongues with large pads that are mainly flipped rather than projected during feeding. Finally, the Bolitoglossini (*Batrachoseps*, *Bolitoglossa*, *Hydromantes*, *Oedipina*, *Nototriton*, *Thorius*) all lack aquatic larvae and have tongues that are free or mainly free at the front and are very highly projectile. This last group includes over 40% of all living species of urodeles.

In an earlier publication (Wake et al., '83) we described patterns of peripheral innervation in lungless salamanders and showed that although there were major differences from group to group in patterns of coiling (to provide additional length for projection) and branching, the fundamental pattern was a conservative one. This conservatism is mirrored in the present study of the central nervous system.

There are three patterns of projection of sensory nerves to the brainstem involved in feeding. The first includes projections from posterior lateral line components associated with IX (Fig. 4). These are encountered only in desmognathines and hemidactyliines in both larvae and adults, and since this projection is not related to terrestrial tongue feeding, it will not be discussed further in this context. We found no remnants of this system in the plethodontines and bolitoglossines, all of which presumably lost aquatic larvae millions of years ago (Wake, '66).

The second pattern includes the superficial, somatosensory projections to the tractus descendens nervi trigemini and involves the ramus hyomandibularis of VII and both IX and X. As seen in Figure 6 (solid bands), these superficial projections are highly overlapping with each other and with the ascending sensory projections of the second spinal nerve. Decussating somatosensory fibers are found mainly in the projections of the r. hyomandibularis of VII and of the second spinal nerve. In contrast to the somatosensory pathway of the trigeminal nerve, which form a broad band of fibers being equally dense from medial to lateral (Roth, unpublished data), superficial pathways of VII, IX, X, and second spinal have more densely arranged fiber bundles at the medial and lateral edges of the band.

The third pattern includes the deeper visceral sensory projections to the fasciculus solitarius from IX and X (Fig. 6, stippled bands). The ramus hyomandibularis of VII does not send fibers to the fasciculus solitarius. The portion from IX is separated into two bundles. Together with that of X it lies deeper than the trigeminal portion of the fasciculus solitarius (Roth, unpublished data). The portion from X related to lateral head and neck musculature forms a remarkable neuropil around the obex and is connected to the commissura infima. Contralateral ascending and descending fibers originate from this neuropil (Fig. 6B); such is not the case with fibers belonging to IX.

There are no sensory projections from XI or first spinal. Apart from the lateral line projections in adult semiaquatic species like *Desmognathus* and *Eurycea*, no major differences were found among the species studied with respect to sensory pathways.

Three types of motor nuclei related to terrestrial feeding are found in plethodontid salamanders. The first type is represented by the combined motor nucleus of VII r. hyomandibularis (VII r.h.), IX, and X (innervation of SARI), which is situated mediolaterally within the gray matter of the brainstem (Fig. 5A). Its neurons have rather narrow dendritic trees that extend mostly ventrolaterally. This arrangement is typical for cranial visceral neurons. This nucleus controls mouth opening (VII r.h.) and tongue protraction (IX/X).

The second type is represented by the motor nucleus of the first spinal/hypoglossal nerve which, together with the nucleus of the second spinal nerve, controls tongue retraction. In all plethodontids this nucleus is situated ventromedially, in front of and behind the obex, at the medial edge of the bulbar and cervical spinal gray matter, and lies significantly more medially and ventrally than the VII/IX/X nucleus (Fig. 7). In the bolitoglossine plethodontids the nucleus consists of one type of neurons with pear-shaped somata and very narrow dendritic trees that extend almost horizontally to the surface (Fig. 5B). These neurons are different from those found in the nuclei of second spinal and spinal accessory nerves with respect to their dendritic arborization, which is rather wide in the latter (Fig. 5C,D). In contrast, in the desmognathine, hemidactyliine, and plethodontine species investigated the hypoglossal nucleus has two types of neurons, one with medially situated pear-shaped somata and medium-wide dendritic trees, and another with laterally situated, spindle-shaped somata and very large dendritic trees (Fig. 5E). These two types of neurons are similar to the two types of neurons found in the nuclei of the second spinal and spinal accessory nerves of the same species (Fig. 5F,G).

This means that in the nonbolitoglossines the nucleus of the first spinal/hypoglossal nerve has a much more spinal appearance than that of the bolitoglossines. The nucleus of the first spinal/hypoglossal nerve in the Bolitoglossini, therefore, has to be considered as a cranial nucleus, whereas that of the nonbolitoglossines is a more spinal nucleus. This is in good accordance with the fact that in the nonbolitoglossines the ramus hypoglossus is constituted by fibers from both the first and second spinal nerves, as is the case in all nonplethodontid salamanders investigated so far, whereas in the Bolitoglossini it is formed by fibers from the hypoglossal nucleus only (Roth et al., '84), and is thus more similar to the situation found in amniotes (which certainly was independently derived).

The third type of motor nuclei is represented by that of the second spinal and the spinal accessory nerve. Whereas in the Bolitoglossini these two nuclei are constituted mostly by neurons with pear-shaped or polygonal somata and wide arborization that form an irregularly structured elongate cluster (Figs. 3B,D, 5C,D), in the other plethodontid groups these nuclei are formed by a medial row of pear-shaped neurons and a lateral row of large spindle-shaped neurons (Figs. 3C,E,F, 5F,G). This latter cytoarchitecture is typical for frog spinal motor nuclei (Székely, '76; Székely and Czeh, '76). It also shows that the spinal accessory is indeed a spinal nerve, despite its cranial exit.

This distinction between plethodontids with and without tongue projection in the cellular components of the hypoglossal, accessory, and second spinal nuclei is also found in the third and fourth spinal nuclei (Roth, unpublished observation) which, to our knowledge, are not involved in the control of the tongue. The difference must, therefore, be regarded as a more fundamental one regarding the cytoarchitecture of cervical spinal motor nuclei. It is still unclear whether it is functionally correlated with differences in feeding behavior. Those groups with two distinctive cell types (e.g., *Plethodon*) are very active and typically couple a strong forward movement of the body with the mouth-opening/tongue-flipping or -projecting phase. In contrast, the bolitoglossini (e.g., *Bolitoglossa* and *Batrachoseps*) usually remain motionless during the phase of tongue projection. The large ventrolateral cells, absent in bolitoglossines,

may be involved in the control of rapid limb and neck movement. This is in accordance with the functional organization of cervical spinal motor nuclei in other amphibians (Székely, '76; Székely and Czeh, '76).

Comparison to nonplethodontid urodeles

Among nonplethodontid urodeles studies exist on the anatomy of the brainstem of *Ambystoma mexicanum* (Opdam and Nieuwenhuys, '76) and unpublished HRP experiments of Roth and Wake on sensory tracts and motor nuclei related to feeding in *Salamandra salamandra* and *Notophthalmus viridescens*. The study of Opdam and Nieuwenhuys on *Ambystoma* was based on Nissl and Bodian material so that an exact determination of size, shape, and architecture of motor nuclei was not possible. Opdam and Nieuwenhuys described the IX/X motor nucleus as a single cell mass of medium-sized cells (about 21 μm in diameter) that extends from the abducens root to the most caudal part of the rhombencephalon. This corresponds in our material to the extent of the combined IX/X nucleus, plus all vagus efferents related to parasympathetic functions.

Opdam and Nieuwenhuys ('76) were unable to confirm the existence of a nucleus nervi facialis pars caudalis, described previously by Herrick ('48) and Leghissa ('49), within the most rostral part of the IX/X nucleus. Our material, however, confirms the existence of such a nucleus, related to the r. hyomandibularis of the facial nerve, but situated in the medial and caudal part of the combined nucleus. Considering the full extent of the nucleus of X, however, the hyomandibular part is rostral in position.

In 15 brains of *Salamandra salamandra* studied with the same HRP technique as used in the plethodontid study presented here, the authors found more or less the same situation as in the plethodontid salamanders, with the greatest similarities to *Plethodon*. In *Salamandra*, too, the motor nuclei of the hypoglossal and second spinal nerves are shorter and wider than in the bolitoglossines, and they show laterally situated spindle-shaped motor neurons, although not as large in size as in *Plethodon*. The rostral branch of the fasciculus solitarius of the IX nerve seems to consist of fewer fibers and to terminate somewhat earlier in *Salamandra* than in the Plethodontidae.

Neither *Salamandra* nor *Notophthalmus* showed an accessorius spinalis. When the last root of the IX/X root complex was labeled, fibers terminated near the level of their exit in neurons that seem to belong to the caudal part of the vagus motor nucleus. No fibers running caudad to the nucleus of the second spinal nerve, as is the case in all plethodontids, could be found. Furthermore, even when rather good labeling of the second spinal nucleus was present, which in plethodontids is usually combined with a staining of the spinal accessory because of opening of the neck musculature and contamination with HRP, no such nerve could be found in *Salamandra* and *Notophthalmus*. However, this does not disprove the existence of a spinal accessory nerve in salamandrids, particularly because HRP experiments with the nerves and nuclei in *Salamandra* and *Notophthalmus* yielded results that often did not have the same quality as in most plethodontids.

Although we have been unable to demonstrate the spinal accessory nerve and nucleus in salamandrids, we suspect its presence is a primitive feature of urodeles and not a novelty restricted to plethodontids. The structure of the system is so similar to that in mammals that it seems highly unlikely that it would have evolved independently

with such unusual common features. We propose to investigate this matter more fully by examining ambystomatid, dicamptodontid, and hynobiid salamanders. All previous authors (e.g., Drüner, '01, '04; Fox, '54; Francis, '34; Norris, '08; Herrick, '48) have included the spinal accessory with the vagus, when it has been mentioned at all, because the techniques used were incapable of demonstrating the total independence of the spinal accessory and vagus motor nuclei.

Comparison with anurans

In contrast to the situation found in urodeles, several studies exist on the distribution of sensory tracts and motor nuclei of the visceral and cervical spinal nerves in *Rana esculenta* (Székely, '76; Székely and Czeh, '76; Nieuwenhuys and Opdam, '76; Matesz and Székely, '77, '78), *Rana pipiens* (Stuesse et al., '83, '84), and *Xenopus laevis* (Nikundiwe et al., '82; Nikundiwe and Nieuwenhuys, '83).

In *Rana esculenta*, Matesz and Székely ('78) and in *Rana pipiens*, Stuesse et al. ('84) find a clear separation between IXth and Xth motor nuclei. The glossopharyngeal nucleus includes about 40 neurons and is situated at the level of the IXth root. The vagal nucleus extends from the mid-myelencephalon (level of Xth root) to the lower myelencephalon and is located at the ventrolateral edge of the periventricular gray matter. This situation in *Rana* corresponds generally to the situation found in plethodontid salamanders as described above. Nikundiwe and Nieuwenhuys ('83) localize the IX/X nucleus of *Xenopus* between the entrance of the posterior lateral line nerve (which corresponds with the first root of the IX/X root complex) and the beginning of the spinal cord. The authors describe loosely arranged cells of medium size (about 19 μm).

In *Rana esculenta* (Matesz and Székely, '77) and *Rana pipiens* (Stuesse et al., '83) the hypoglossal nucleus consists of two groups of neurons, a medial and a lateral one. The medial one is situated around the obex and overlaps with the Xth nucleus. The lateral group extends somewhat more caudally and is continuous with efferent cell columns in the cervical spinal cord. As in plethodontids, the dendrites extend primarily in a lateral direction and intersect with the fibers of the lateral funiculus. A second branch of dendrites extends in a dorsal direction, terminating in the nucleus fasciculi solitarii. Stuesse et al. ('83) report that in *Rana pipiens* the hypoglossal nucleus contains a small number of afferents that enter the tractus descendens nervi trigemini and descend into Lissauer's tract. In contrast, in all plethodontids studied so far, as well as in other urodeles, we found the hypoglossal nerve to be strictly motor.

Matesz and Székely ('77) report that in *Rana esculenta* only the medial cell group innervates tongue musculature in frogs and they consider this group to be the hypoglossal nucleus proper. The two rows of cells found in such plethodontids as *Plethodon*, *Eurycea*, and *Desmognathus* may be the homologues of the two hypoglossal nuclei in frogs. The lateral cell group in both salamanders and frogs may be involved with neck and body rather than with tongue movement. Only this suggestion is consistent with the absence of a distinct lateral cell group in bolitoglossines.

Matesz and Székely ('78) describe an accessorius motor nucleus in *Rana esculenta*. This nucleus is situated around the obex, dorsolateral to the hypoglossal nucleus. Despite this location it contains neurons that show a multipolar shape and wide dendritic arborization typical for spinal

motor neurons and is, therefore, considered by the authors as a true *spinal accessorius*.

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