

Organization of the Motor Nuclei in the Cervical Spinal Cord of Salamanders

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ABSTRACT

The distribution and cytoarchitecture of motor nuclei of the cervical spinal cord were studied by using HRP techniques (whole mounts and sections) in 22 species of salamanders (families Hynobiidae, Dicamptodontidae, Ambystomatidae, Salamandridae, and Plethodontidae) representing a wide variety of life histories and functional modes of feeding. The nucleus of the first spinal nerve extends from the level of, or slightly caudad to, the root of the tenth cranial nerve, almost to the ventral root of the second spinal nerve. Approximately one-half of this nucleus is situated in the brainstem. This anterior extension is longest in bolitoglossine plethodontids. The nucleus of the second spinal nerve extends from the root of the first spinal nerve to the dorsal root of the second spinal nerve.

The nuclei of the first and second spinal nerves in all species except bolitoglossines have motor neurons arranged in two columns: a lateral one containing large spindle-shaped cells and a medial one containing pear-shaped or polygonal smaller cells. The primary dendrites of these lateral and medial cells are parallel and their arborization is relatively narrow. In contrast, bolitoglossines lack the lateral motor column. The nucleus of the first spinal nerve consists only of a medial band of pear-shaped and sometimes polygonal cells, and the nucleus of the second spinal nerve is a wider band of pear-shaped and polygonal cells which are always situated inside the periventricular gray matter. The arrangement of the somata in bolitoglossines is less organized and the primary dendrites are less parallel and have a broader arborization than in other salamanders. In all species, cells in the second spinal nucleus are arranged in a less orderly manner than those in the first.

All salamanders studied possess a spinal accessory nerve whose motor neurons are located in the cervical spinal cord; the axons leave the brainstem with fibers of the vagus nerve. The rostrocaudal extent of this nucleus differs markedly among species. In bolitoglossines the nucleus is more or less restricted to the region of the nucleus of the second spinal nerve. In all other species studied, the accessory nucleus extends from the obex to the caudal end of the nucleus of the third spinal nerve. In the tribe Plethodontini the cytoarchitecture of the accessory nucleus is similar to that of the second spinal. In desmognathine and hemidactyliine plethodontids as well as in all nonplethodontid species studied the nucleus consists of pear-shaped and cone-shaped cells. No separate lateral motor column is present and the cone-shaped cells are found at various positions inside and slightly outside the gray matter. In bolitoglossines both pear-shaped and polygonal cells are found in the gray matter; again, no lateral motor column is present.

A developmental-evolutionary hypothesis is presented to account for the unique pattern of cellular organization in the bolitoglossines, and functional and phylogenetic implications of our findings are considered.

Key words: motor neurons, spinal accessory, HRP

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In salamanders, the nuclei of the cervical spinal cord play important roles in feeding and visuomotor behavior. The first and second spinal nerves control retraction of the tongue, and they are also involved in aiming during tongue projection (Roth and Wake, '85a). The spinal accessory nerve controls action of the neck musculature involved in feeding and in optomotor behavior (Manteuffel and Naujoks-Manteuffel, '87).

In an earlier paper we reported two different patterns of spinal cord organization in salamanders of the family Plethodontidae (Roth and Wake, '85a). In some genera (e.g., *Plethodon*) we found distinct medial and lateral groups of cells. In other genera (e.g., *Batrachoseps*) we found only a single column of cells in a medial position. The situation in salamanders other than members of the family Plethodontidae and the developmental basis for differences in motor neuron morphology remained unclear. We also demonstrated the existence of a spinal accessory nerve and nucleus in plethodontids, with neurons situated in the cervical spinal cord and axons leaving the brain via the brainstem (Roth et al., '84). However, it remained unclear whether salamanders other than plethodontids possessed this nerve, whose existence as a discrete entity was denied by many authors (e.g., Francis, '34).

We here report new observations on a greater diversity of species, including members of the families Hynobiidae, Salamandridae, Ambystomatidae, Dicamptodontidae, and Plethodontidae. In order to investigate the role of differences in ontogeny and life history, we have included species that have an aquatic larval stage as well as those that have abandoned the larval stage and have direct development on land. We also have included a wide variety of feeding modes in our sample to see what, if any, differences in distribution and cytoarchitecture of the cervical spinal motor nuclei correlate with differences in mode of feeding.

MATERIALS AND METHODS

We studied members of the salamander families Hynobiidae (*Salamandrella keyserlingii*, n = 1), Dicamptodontidae (*Dicamptodon ensatus*, n = 1; *Rhyacotriton olympicus*, n = 6), Ambystomatidae (*Ambystoma gracile*, n = 1; *A. macrodactylum*, n = 3), Salamandridae (*Salamandra salamandra*, n = 5; *Salamandrina terdigitata*, n = 10), and Plethodontidae (Desmognathinae: *Desmognathus aeneus*, n = 1; *Des. monticola*, n = 1; *Des. ochrophaeus*, n = 7; *Des. wrighti*, n = 4. Plethodontinae, Hemidactyliini: *Eurycea bislineata*, n = 3; *Gyrinophilus porphyriticus*, n = 2; *Pseudotriton ruber*, n = 1; Plethodontini: *Aneides flavipunctatus*, n = 1; *Plethodon dunni*, n = 3; *Pleth. jordani*, n = 12; Bolitoglossini: *Batrachoseps attenuatus*, n = 10; *Bolitoglossa subpalmata*, n = 7; *Nototriton picadoi*, n = 1; *Oedipina uniformis*, n = 1; *Hydromantes italicus*, n = 8). We used methods described in detail in earlier papers (Roth and Wake, '85a; Roth et al., '84). Specimens used in the experiments were anesthetized both prior to surgery and, subsequently, prior to perfusion, by immersion in a 1% aqueous solution of tricaine-methanesulfonate (MS-222) for from 1 to 5 minutes, depending on the size of the specimen and the species. HRP (Boehringer, grade I; Serva 540 µg) was applied as minute pellets carried on the tips of micropipettes, which were applied for 10–15 minutes to the cut

ends of selected nerves, both peripherally and near the brain, in order to fill individual nerve cells and their projections. The nerve stumps were isolated and kept relatively dry to retard the diffusion of HRP. The sites of application of HRP were as follows (Fig. 1 in Roth et al., '88, this volume). *First spinal nerve*: 1) intermandibular region in front of gular fold and 2) between anastomosis with second spinal nerve and brain. *Second spinal nerve*: between anastomosis with first spinal nerve and brain. *Spinal accessory nerve*: 1) between innervation of neck musculature and IX-X ganglion and 2) in the IX-X ganglion. Following a recovery period of 48–72 hours, the specimens were treated with diaminobenzidine as whole mounts or as frozen sections. Whole mounts were subsequently cleared in methyl salicylate for visualization of details of cellular organization. Some of these whole mounts were returned to alcohol and following standard procedures were embedded in Epon and cut in transverse sections of 30 µm.

RESULTS

Distribution of roots and pattern of peripheral innervation

The general organizational pattern of the brainstem and cervical spinal cord in relation to cranial nerves V–X and to cervical spinal nerves is shown in Figure 1 (double arrows) of Roth et al. ('88, this volume).

In all adult salamanders studied so far the first spinal nerve has only a ventral and purely motor root; a dorsal sensory root is lacking. The root is situated slightly caudal to the obex of the rhombencephalon and usually consists of three or four rootlets. The second spinal nerve is the first spinal nerve with both dorsal and ventral roots and a dorsal ganglion. There is an anastomosis near the brain between the first and the second spinal nerves which varies greatly in length and diameter among species. It carries motor

Abbreviations

A	axons
CB	cerebellum
FK	knee of the facialis nerve
SS	somatosensory fibers
SScl	contralateral somatosensory fibers
TO	Tectum opticum
V	Trigeminus nerve/nucleus
Va	anterior portion of the trigeminus nucleus
Vp	posterior portion of the trigeminus nucleus
VI	abducens nerve/nucleus
VIacc	accessory nucleus of the abducens nerve
VII	facialis nerve/nucleus
VIIa	anterior portion of the facialis nucleus
VIIp	posterior portion of the facialis nucleus
VII hm	separate motor root of the ramus hyomandibularis of the facialis nerve
IX	glossopharyngeus nerve/nucleus
X	vagus nerve/nucleus
Xm	motor root of the vagus nerve
XPS	parasympathetic portion of the vagus nucleus
XSAR	portion of the vagus nucleus supplying the subarcualis rectus muscle
XI	accessorius spinalis nerve/nucleus
1SP	first spinal nerve/nucleus
2SP	second spinal nerve/nucleus
2SPm	motor root of the second spinal nerve

fibers from the second spinal nerve running to the m.rectus cervicis profundus and superficialis (Roth and Wake, '85a). In most nonbolitoglossine salamanders, fibers from the first and second spinal nerves together constitute the ramus hypoglossus that innervates the following muscles (from caudal to rostral): the geniohyoideus medialis, the supra-peduncularis (if present), the geniohyoideus lateralis, and the genioglossus (if present); a medial branch bifurcates at different levels of the lower jaw from the hypoglossus, enters the tongue, and innervates the tongue pad (Wake et al., '83). In bolitoglossine salamanders the ramus hypoglossus is exclusively constituted by the first spinal nerve; thus, the muscles mentioned are innervated by the first spinal nerve alone. In addition the first and second spinal nerves separately innervate the rectus cervicis profundus and superficialis and parts of the neck musculature.

The spinal accessory nerve is purely motor, and its fibers exit the brain through the last rootlets of the IX-X-XI root complex at the level of or slightly rostral to the obex. Its

fibers contribute to the innervation of the cephalodorsosub-pharyngeus and the cucullaris muscles of the neck.

Distribution of motor nuclei in the cervical spinal cord

In bolitoglossine plethodontid salamanders the first spinal nucleus begins at or just posterior to the entrance of the second root of the IX-X-XI nerve complex (Fig. 1A). In all other salamanders the motor nucleus of the first spinal nerve begins more posteriorly, near the midpoint between the second and third roots of the IX-X-XI complex (Fig. 1B). The nucleus ends slightly behind the midpoint between the first and second spinal roots in all species studied. The rostral part of the first spinal nucleus overlaps with parts of the motor nuclei of VII and X and in the Bolitoglossini also with the nucleus of VI (Fig. 1).

The motor nucleus of the second spinal nerve arises slightly rostral to the midpoint between the first and second spinal roots. The nucleus ends near the midpoint between

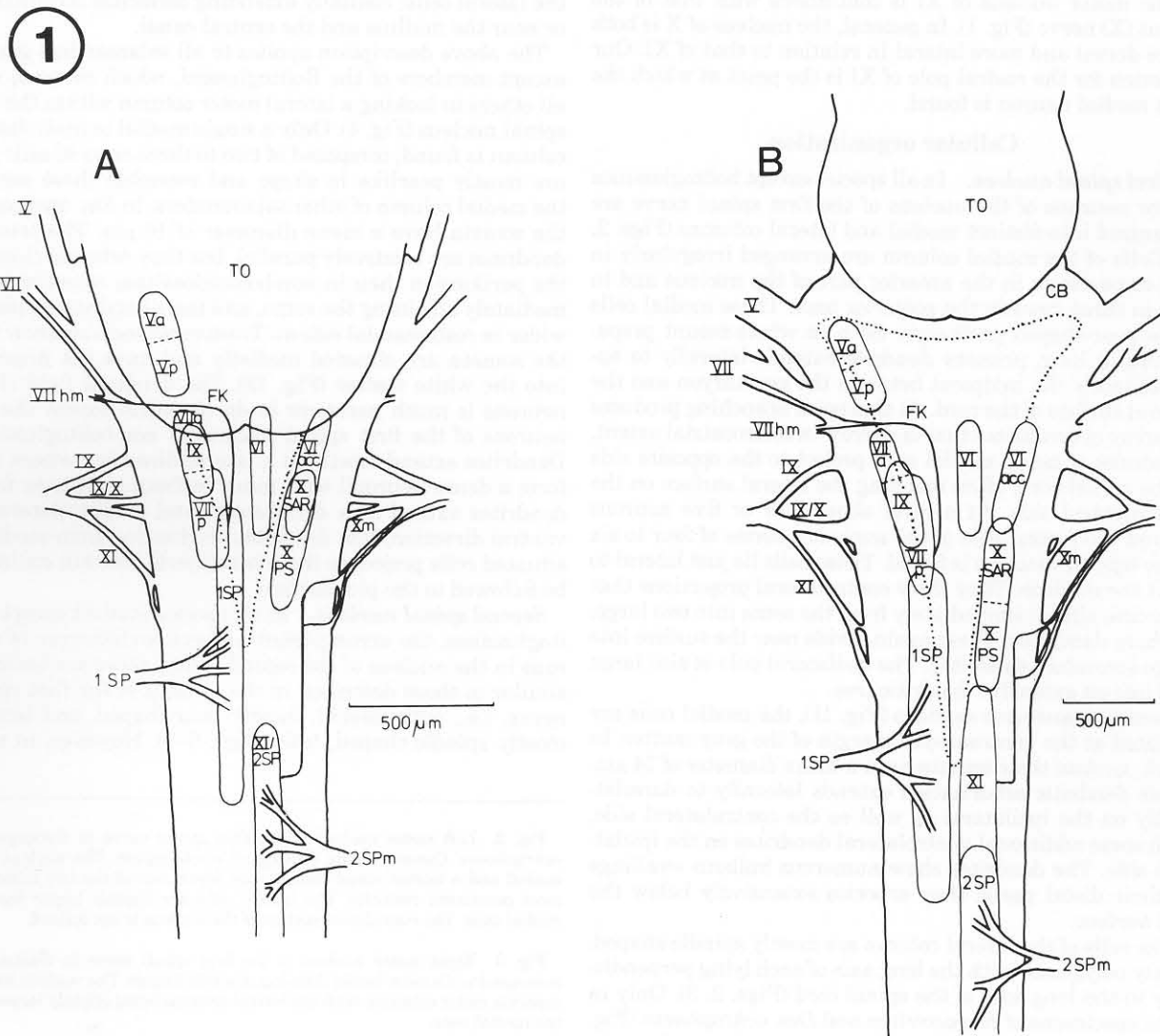


Fig. 1. The topological relationships of the motor nuclei in the brainstem and cervical spinal cord in (A) bolitoglossine salamanders (Plethodontidae: Bolitoglossini, based on *Batrachoseps attenuatus*) and (B) nonbolitoglossine

plethodontid salamanders and salamanders in the families Ambystomidae, Dicamptodontidae, Hynobiidae, and Salamandridae (based on *Salamandra salamandra*).

the ventral roots of the second and third spinal nerves at the level of the dorsal root of the second spinal nerve (Fig. 1). Thus, the motor nuclei of the first and second spinal nerves overlap by about one-fourth of their total length.

The position of the rostral pole of the nucleus of the spinal accessory nerve (cranial nerve XI) varies among species. In all salamanders except bolitoglossines the nucleus begins at the level of the first spinal root and extends at least to the level of the dorsal root of the second spinal nerve, often extending to the level of the dorsal root of the third spinal nerve (Fig. 1B). In bolitoglossines this nucleus begins more posteriorly, approximately at the midpoint between the first and second spinal roots (Fig. 1A). It ends at or near the posterior end of the second spinal nucleus and thus is substantially shorter than the nucleus in other species. The nucleus of the spinal accessory nerve completely overlaps that of the second spinal nerve, partially overlaps that of the first spinal nerve, and in all species but the bolitoglossines it also partially overlaps that of the third spinal nerve.

The motor nucleus of XI is continuous with that of the vagus (X) nerve (Fig. 1). In general, the nucleus of X is both more dorsal and more lateral in relation to that of XI. Our criterion for the rostral pole of XI is the point at which the first medial neuron is found.

Cellular organization

First spinal nucleus. In all species except bolitoglossines motor neurons of the nucleus of the first spinal nerve are organized into distinct medial and lateral columns (Figs. 2, 3). Cells of the medial column are arranged irregularly in one or two rows in the anterior part of the nucleus and in two to three rows in the posterior part. These medial cells have pear-shaped perikarya. Seen in whole-mount preparations, a long, primary dendrite extends laterally to approximately the midpoint between the perikaryon and the lateral surface of the cord. At this point branching produces an array of dendrites that is narrow in rostrocaudal extent. Dendrites of many medial cells project to the opposite side of the spinal cord, often reaching the lateral surface on the contralateral side. At a level about four or five neurons behind the rostral pole of the nucleus a series of four to six large bipolar neurons is found. These cells lie just lateral to or at the midline. They show contralateral projections that bifurcate almost immediately from the soma into two large, primary dendrites. These again divide near the surface into large secondary dendrites. The ipsilateral pole is also large and has an extensive dendritic tree.

Seen in transverse sections (Fig. 11), the medial cells are situated at the ventromedial margin of the gray matter. In *Pleth. jordani* their somata have a mean diameter of 14 μm . Their dendritic arborization extends laterally to dorsolaterally on the ipsilateral as well as the contralateral side, with some additional ventrolateral dendrites on the ipsilateral side. The dendrites show numerous bulbous swellings in their distal parts; they arborize extensively below the pial surface.

The cells of the lateral column are mostly spindle-shaped, rarely polygonal, with the long axis of each lying perpendicular to the long axis of the spinal cord (Figs. 2, 3). Only in some specimens of *Rhyacotriton* and *Des. ochrophaeus* (Fig. 2) and in juvenile *Plethodon* have we found more than one row of cells in the lateral column. Alignment of the cells in the lateral column is more regular than in the medial column, but the position of the neurons varies more in the

posterior half of the nucleus than in the anterior half. Two to three thick dendritic branches arise from the lateral end of each perikaryon, and one or two smaller dendrites emerge from the medial end. While somata of the cells in the lateral column are usually somewhat larger than those of the medial column, in *Pleth. jordani* the lateral somata are much larger than the medial ones with mean diameters of 25 μm along their long axis. The separation of the two columns is most distinct in the genera *Plethodon* and *Desmognathus*.

Seen in transverse sections (Fig. 11), the lateral cells have clearly migrated into the white matter of the cord. The spindle-shaped somata show an oblique dorsolateral-ventromedial orientation. The wide arborization of their dendrites extends from dorsal to ventromedial. Dendritic arborization is greatest laterally, below the pial surface, more or less exactly below the dorsoventral level of the entrance of the 11th nerve root. Dendrites show numerous swellings in their distal parts, especially near the surface of the cord. Dendrites crossing the midline could not be discovered for the lateral cells; medially extending dendrites terminate at or near the midline and the central canal.

The above description applies to all salamanders studied except members of the Bolitoglossini, which contrast with all others in lacking a lateral motor column within the first spinal nucleus (Fig. 4). Only a single medial to mediolateral column is found, composed of two to three rows of cells that are mostly pearlike in shape and resemble those seen in the medial column of other salamanders. In *Bat. attenuatus*, the somata have a mean diameter of 16 μm . The primary dendrites are relatively parallel, but they arborize closer to the perikaryon than in non-bolitoglossines, sometimes immediately adjoining the soma, and their dendritic fields are wider in rostrocaudal extent. Transverse sections show that the somata are situated medially and have not migrated into the white matter (Fig. 12). The dendritic field of the neurons is much narrower in dorsoventral extent than in neurons of the first spinal nucleus of non-bolitoglossines. Dendrites extend mostly in a lateral direction, where they form a dense neuropil with many bulbous swellings; fewer dendrites extend in a dorsolateral and a ventrolateral-to-ventral direction; fine dendrites originating from medially situated cells project to the contralateral side but could not be followed to the pial surface.

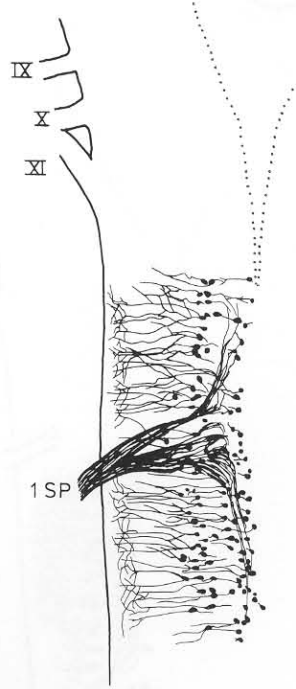
Second spinal nucleus. In all species studied except bolitoglossines, the arrangement and cytoarchitecture of neurons in the nucleus of the second spinal nerve are basically similar to those described in the nucleus of the first spinal nerve, i.e., with medial, mostly pear-shaped, and lateral, mostly spindle-shaped, cells (Figs. 5-7). However, in most

Fig. 2. Left motor nucleus of the first spinal nerve in *Desmognathus ochrophaeus*. Camera lucida drawing of whole mount. The nucleus has a medial and a lateral motor column; the separation of the two columns is most prominent rostrally. The lateral cells are slightly larger than the medial ones. The rostralmost portion of the nucleus is not stained.

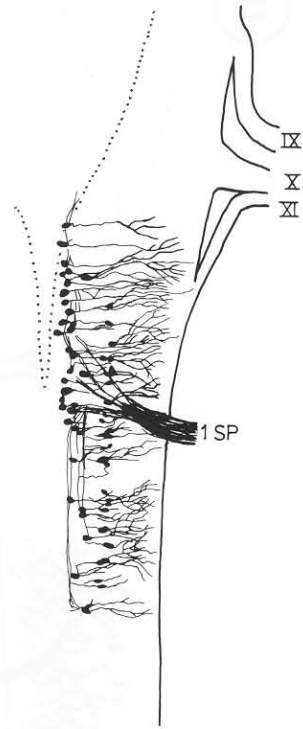
Fig. 3. Right motor nucleus of the first spinal nerve in *Salamandra salamandra*. Camera lucida drawing of whole mount. The nucleus has two separate motor columns, with the lateral neurons being slightly larger than the medial ones.

Fig. 4. Left motor nucleus of the first spinal nerve in *Hydromantes italicus*. Camera lucida drawing of whole mount. The nucleus has one medially situated column of pear-shaped cells. Note contralateral projections of cells in rostral part of nucleus.

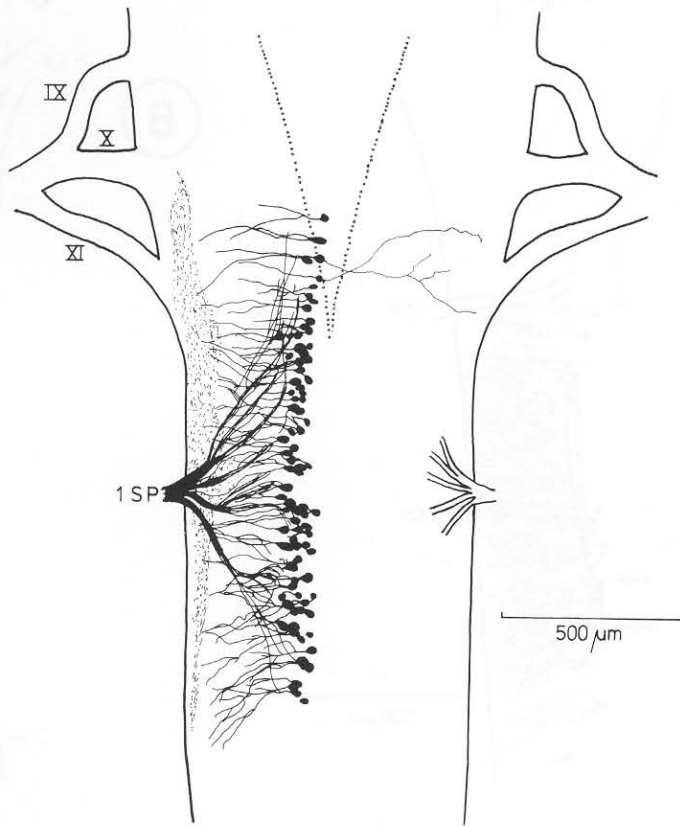
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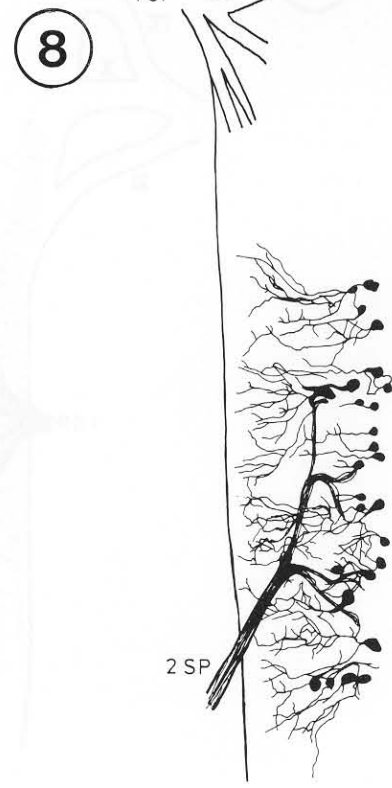
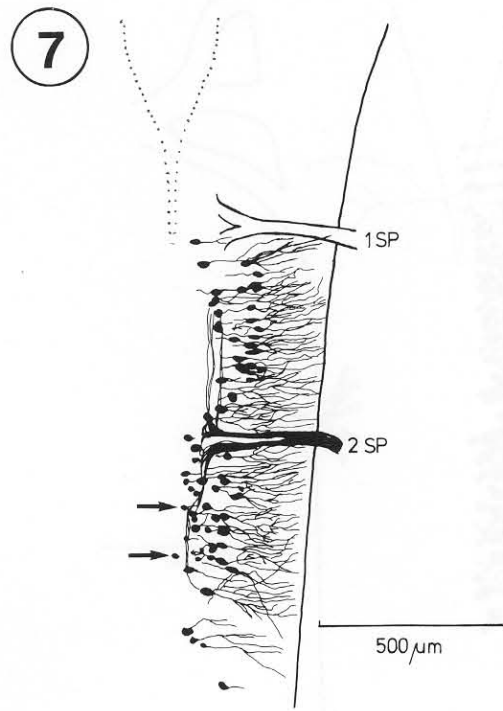
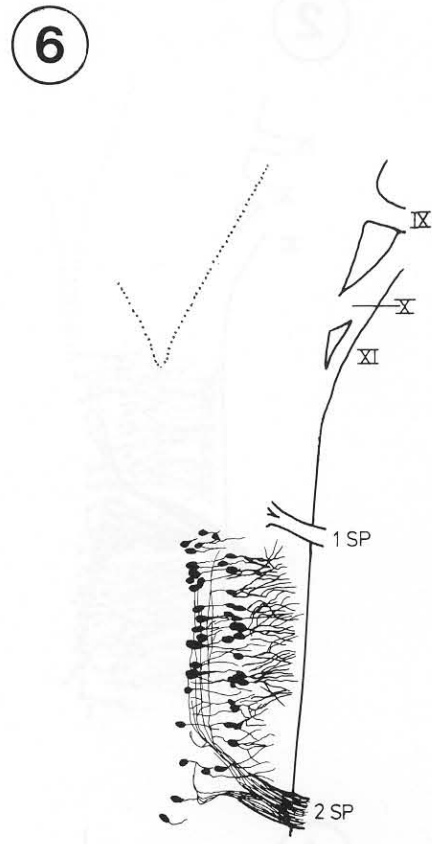
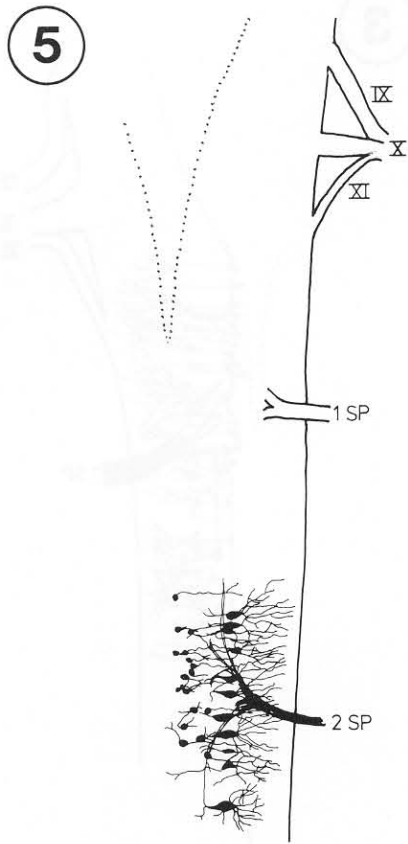


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cases the arrangement of the cells is less organized and the separation of the lateral and medial motor column is less clear. In the caudal portion of the nucleus, the two columns often tend to merge (Fig. 7).

In transverse sections, the medial pear-shaped cells show a dendritic arborization that is similar to that found in the first spinal nerve although the contralaterally projecting dendrites are usually finer. In *Pleth. jordani* the mean diameter of the somata of the medial cells is 17 μm ; in *R. olympicus* it is 21 μm . In *Plethodon*, the spindle-shaped neurons show again a clearly migrated lateral position within the white matter (Fig. 13). They show a wider dendritic arborization than the lateral cells of the first spinal nerve. Dorsally, dendrites project into the region of the ascending and descending sensory tracts of the spinal nerves as well as the descending sensory tracts of cranial nerves V, VII, IX, and X. Massive dendritic projections are found in dorsolateral, ventrolateral, and ventromedial directions, with numerous bulbous swellings at their more distal parts. In *Rhyacotriton* (Fig. 14), both spindle-shaped and polygonal cells are found, the latter generally less migrated into the white matter. The mean diameter of the somata of the lateral neurons is 29 μm in *Plethodon* and 28 μm in *Rhyacotriton*.

In the bolitoglossines, a lateral motor column is absent as in the nucleus of the first spinal nerve (Fig. 8). However, the arrangement of cell somata is more disorganized and the cytoarchitecture is more complex than in the first spinal nerve. In addition to medial pear-shaped cells, with a mean diameter of 17 μm in *Batrachoseps* (Fig. 15), polygonal cells are found, often at more lateral positions, but always within the gray matter and without any special alignment. These neurons are somewhat larger in size (mean diameter of 20 μm in *Batrachoseps*) and have two or three primary dendrites originating from the soma. These dendrites display wide dendritic arborization both in rostrocaudal and dorsoventral planes. In the brains of the largest and oldest bolitoglossines (Fig. 8) the highest degree of morphological complexity is found, consisting of pear-shaped and cone-shaped somata. These cells lack an orderly arrangement.

In transverse sections, the cells of the second spinal nerve in bolitoglossines again show no sign of migration into the white matter (Fig. 15). However, they display generally the same dendritic arborization pattern as those of the nonbolitoglossines. The medially situated neurons show dendrites

extending mostly in lateral, ventrolateral, and ventromedial directions, and they also project to the contralateral side. More laterally situated neurons show wider arborization, with dendrites making contact with dorsomedial and dorsolateral sensory tracts.

Stainings of more caudal spinal nerves and related nuclei reveal the same differences in cytoarchitecture as those observed in the second spinal nucleus in bolitoglossines and all other species, respectively.

Spinal accessory nucleus. The cytoarchitecture of the spinal accessory nucleus is more variable among species than is that of other cranial or spinal motor nuclei. Three types of cytoarchitecture can be distinguished.

The first type of cytoarchitecture, found only in the tribe plethodontini, is more or less identical to that found in the first and second spinal nerves of nonbolitoglossine salamanders, with medial pear-shaped and lateral spindle-shaped cells. The latter cells are located in the white matter.

The second type occurs in desmognathine and hemidactyliine members of the Plethodontidae (Fig. 9), as well as in *Salamandra*, *Ambystoma*, and *Salamandrella*. In the Plethodontids and *Salamandrella* no separate lateral motor column exists and no spindle-shaped cells are found. There are small pear-shaped cells that lie close to the midline and larger cells that have a very thick, cone-shaped primary dendrite from which rather thin dendrites originate that arborize most heavily in lateral and ventrolateral, and less heavily in ventromedial, directions (Fig. 16). A few dendrites extend dorsally and make contact with the dorsolateral somatosensory tracts. These cone-shaped cells are variable in their rostrocaudal orientation and are found both medially and more laterally within and slightly outside the gray matter.

In *Salamandra* and *Ambystoma* the situation is similar to that found in desmognathines and hemidactyliines, but a few spindle-shaped cells are located away from the midline, and there are in addition some medial pear-shaped cells as well as some medial and lateral cone-shaped cells.

In bolitoglossine members of the Plethodontidae as well as in *Rhyacotriton* the third type of cytoarchitecture is found. The spinal accessory nucleus contains small medial pear-shaped and larger medial to lateral polygonal cells with no separate lateral motor column. This situation is similar to that found in the nucleus of the second spinal nerve of these salamanders (Fig. 10). The more lateral polygonal cells are never found outside the periventricular gray matter (Fig. 17). These cells have a mean diameter of 20 μm in *Batrachoseps*. Three to four primary dendrites originate from the soma and extend to dorsolateral, lateral, and ventrolateral directions before almost immediately dividing into secondary dendrites that show wide arborization in dorsolateral to ventromedial directions, with a few dorsally extending dendrites. No contralaterally projecting dendrites could be detected; the medial dendrites terminated at or near the midline.

Ontogenetic variation. We investigated the cervical spinal motor nuclei of three recently metamorphosed (ca. 50-mm snout-vent length—SVL) juvenile *A. macrodactylum*. Two individuals display two distinct columns of cells in the first and second spinal motor nuclei—a medial one with mainly small cells (20–30 μm) and a lateral one with exclusively large, spindle-shaped cells (40–50 μm). There are a few spindle-shaped cells in an intermediate position. In one of these animals there are two large, rounded cells (40–50 μm) at the rostral end of the first spinal nucleus in

Fig. 5. Right motor nucleus of the second spinal nerve in *Plethodon jordani*. Camera lucida drawing of whole mount. The nucleus has two columns of motor neurons; the lateral spindle-shaped cells are much larger than the medial pear-shaped ones. The nucleus is incompletely stained both rostrally and caudally.

Fig. 6. Right motor nucleus of the second spinal nerve in *Rhyacotriton olympicus*. Camera lucida drawing of whole mount. The nucleus has two separate columns of motor neurons. Lateral neurons are only slightly larger than medial ones. Only the rostral part of the nucleus is stained.

Fig. 7. Right motor nucleus of the second spinal nerve in *Rhyacotriton olympicus*. Camera lucida drawing of whole mount of a younger animal than in Figure 6. The separation of the nucleus into a medial and a lateral motor column is still incomplete. Very small pear-shaped neurons, situated along the midline (arrows), are thought to be newly born cells.

Fig. 8. Left motor nucleus of the second spinal nerve in *Hydromantes italicus*. Camera lucida drawing of whole mount of a brain from a large, old animal. The nucleus shows an irregular arrangement of widely arborizing neurons. More lateral cells tend to be larger in size.

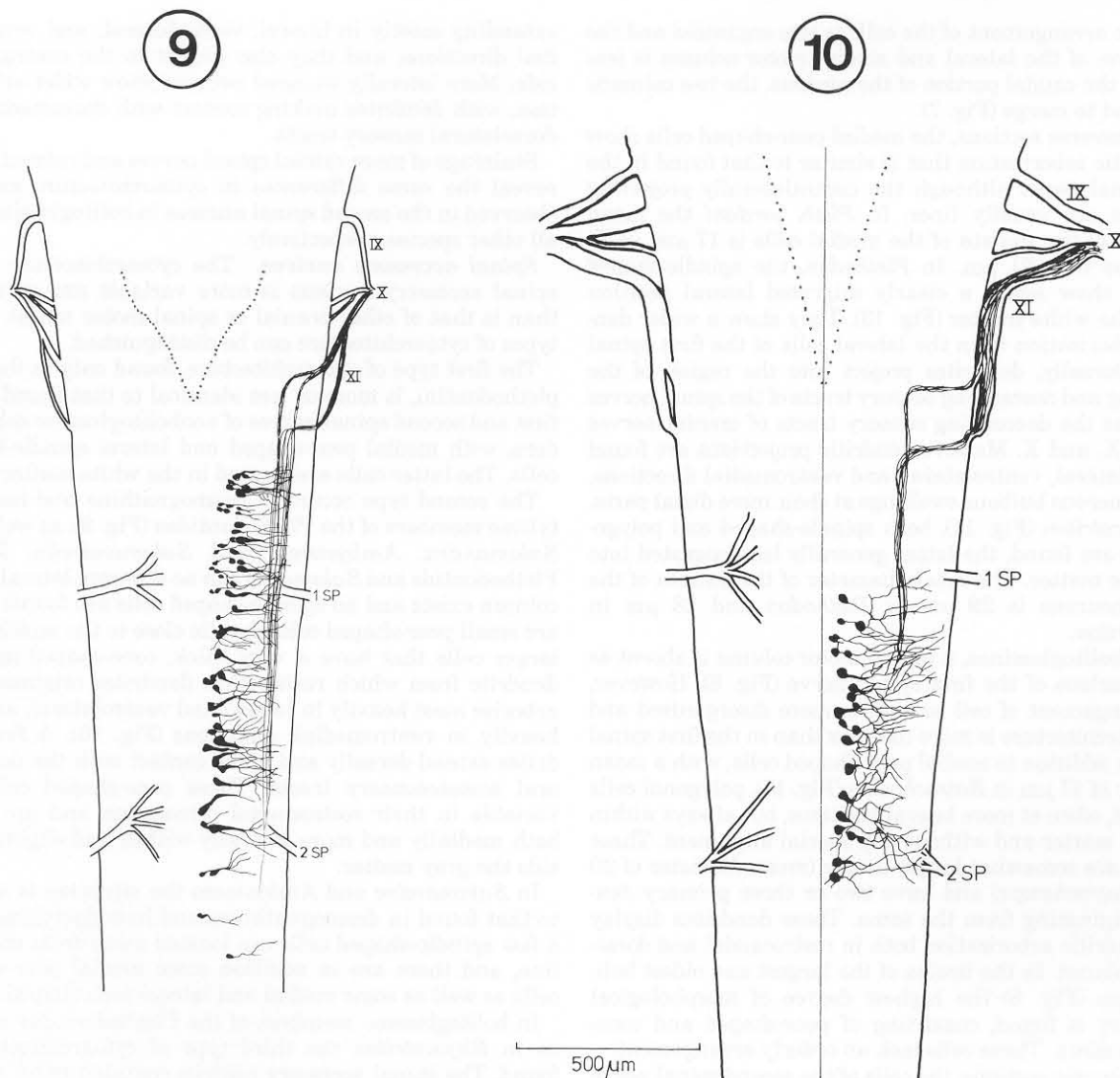


Fig. 9. Left motor nucleus of the spinal accessory nerve in *Eurycea bislineata*. Camera lucida drawing of whole mount. Cells of the nucleus are arranged irregularly. Small pear-shaped perikarya are found mostly in medial positions, while larger cone-shaped perikarya are situated in medial-to-lateral positions. No separation of the two cell types into two columns exists. Note the conspicuous pathway of the axons leaving the brain through several rootlets at the level of the obex.

Fig. 10. Right motor nucleus of the spinal accessory nerve in *Bolitoglossa subpalmata*. Camera lucida drawing of whole mount. Note the irregular arrangement of pear-shaped and polygonal cells. No separation of the cell types into two columns exists. The axons leave the brain through the last two roots of the IX-X-XI root complex at the level of the obex.

a medial position. Each of these cells has two large, laterally oriented processes that bifurcate near the cell body. These processes have a narrow lateral projection. We consider these cells to be primary motor neurons. In the third specimen the second spinal nucleus is relatively narrow (200 μm) and has an unorganized appearance. There are relatively few spindle-shaped cells, and these all are in intermediate or extreme lateral (at the edge of the gray matter) positions.

In *Pleth. jordani* small juveniles (from 17.5 to 39 mm SVL) were studied in addition to adults. Even in the smallest specimens, the nucleus of the first spinal nerve is fully developed with both medial pear-shaped and lateral spindle-shaped cells. However, the nucleus of the second spinal nerve is less developed in that spindle-shaped cells occur in medial, intermediate, and lateral positions. The least de-

gree of morphological differentiation is found in the spinal accessory nucleus. Most cells are in medial and a few are in intermediate positions. Some spindle-shaped cells occur in rather medial positions. No primary motor neurons are found even in the smallest specimens.

In small juveniles (20–21 mm SVL; 3–4 months post-hatching) of *H. italicus* no lateral motor column is present. However, at the anterior end of the nucleus of the first spinal nerve a few spindle-shaped cells are found among the mainly pear-shaped cells. Some of these spindle-shaped cells occupy a somewhat more lateral position. In larger juveniles and adults we have found no spindle-shaped cells, which evidently disappear during ontogeny.

Comparison of three brains of increasing size in *R. olympicus* reveals progressive differentiation. In the smallest brain (Fig. 7) no clear separation of the motor columns is

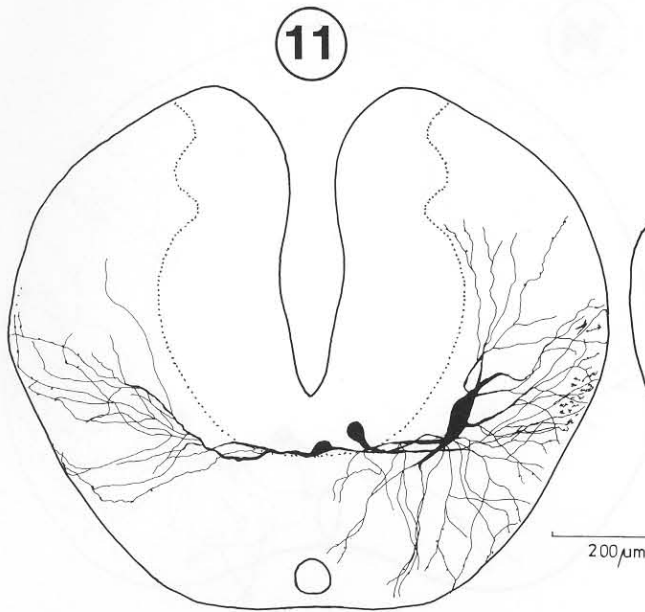


Fig. 11. Camera lucida drawing of a transverse section through the motor nucleus of the first spinal nerve in *Plethodon jordani*. The section shows a large spindle-shaped cell in a lateral position inside the white matter, with widespread dendritic arborization, and small cells in a medial position inside the gray matter. One pear-shaped cell has a dendritic tree projecting to the ipsilateral side, and the bipolar cell has strong contralateral and weaker ipsilateral dendritic projections and a cell body situated at the midline. Along the distal parts of the dendrites many bulbous swellings are found.

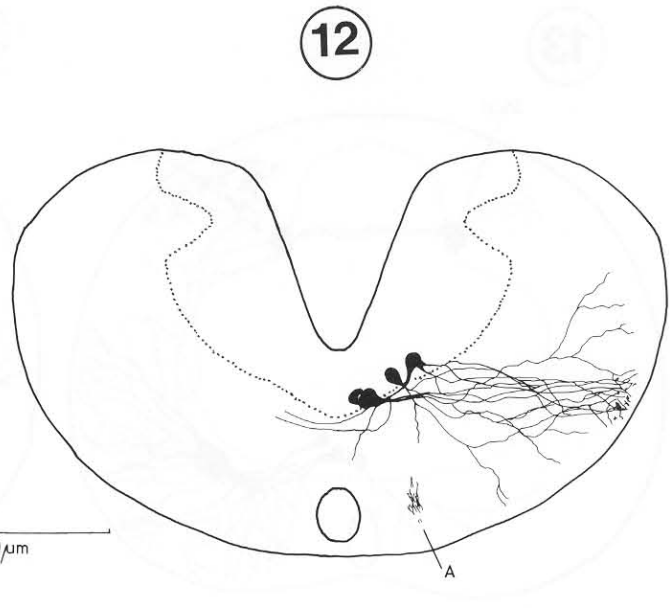


Fig. 12. Camera lucida drawing of a transverse section through the motor nucleus of the first spinal nerve in *Batrachoseps attenuatus*. The section shows several pear-shaped cells that are more medially situated and one polygonal cell that is situated more laterally (but well inside the periventricular gray matter). Note the rather narrow dendritic arborization. Dendrites crossing to the contralateral side appear to fall short of the lateral surface.

seen, and cells are present from medial to lateral positions. Spindle-shaped cells are found both medially and laterally. In the largest brain (Fig. 6) there are two clearly differentiated columns, and spindle-shaped cells are exclusively lateral in position.

DISCUSSION

Important differences were found among species in the topology and cytoarchitecture of motor nuclei in the cervical spinal cord. This result is somewhat surprising, given the relative conservatism of the brainstem in the same species of salamanders (Roth et al., '88).

Representatives of the five families of salamanders investigated share a basic organizational plan for the cervical spinal cord. Discrete first and second spinal nerves and a spinal accessory nerve are found in all stages of the life history of all salamanders we have studied. Earlier we reported that we were unable to find the spinal accessory nerve in members of the family Salamandridae (Roth and Wake, '85a), but we now can document the existence of this nerve in the family. The motor nucleus clearly is present but its roots and rootlets are not as well differentiated as in other families. This result, together with the discovery of the nerve in anurans (Oka et al., '87), strongly suggests that the spinal accessory nerve is an ancestral feature not only of salamanders but perhaps of all tetrapods.

Interspecific differences in topology

The motor nucleus of the first spinal nerve extends rather far forward into the brainstem in all salamanders, but condensation of the region is more extreme in bolitoglossine

plethodontids than in other species (Roth et al., '88). In other respects, the nuclei of the first and second spinal nerves are topologically conservative. The nucleus of the spinal accessory nerve is subject to more variability, especially in its length. This nucleus overlaps the nuclei of the first three spinal nerves in all salamanders except the bolitoglossines, in which it overlaps only with the nucleus of the second and the extreme caudal portion of the first spinal nerves.

Interspecific differences in cytoarchitecture

We recognize three major classes of organization of motor neurons in the nuclei of the cervical spinal cord among salamander taxa. The first is found only in the plethodontid tribe Plethodontini. Here, the first and second spinal nuclei (as well as the third and fourth and perhaps other more caudal spinal nuclei) and the spinal accessory nucleus all have the same cytoarchitecture (a medial column of small, mostly pear-shaped cells, and a lateral column of larger, mostly spindle-shaped cells). The lateral cells are always found well migrated within the white matter of the spinal cord, and they are aligned more or less in an orderly rostrocaudally oriented row.

The second class is found in two plethodontid taxa (subfamily Desmognathine and tribe Hemidactyliini) as well as in all nonplethodontid species studied so far (including members of the families Ambystomatidae, Dicamptodontidae, Salamandridae, and Hynobiidae) except for the dicamptodontid genus *Rhyacotriton*. The first and second spinal motor nuclei of these groups have the same cytoarchitecture as the plethodontini, but the spinal accessory

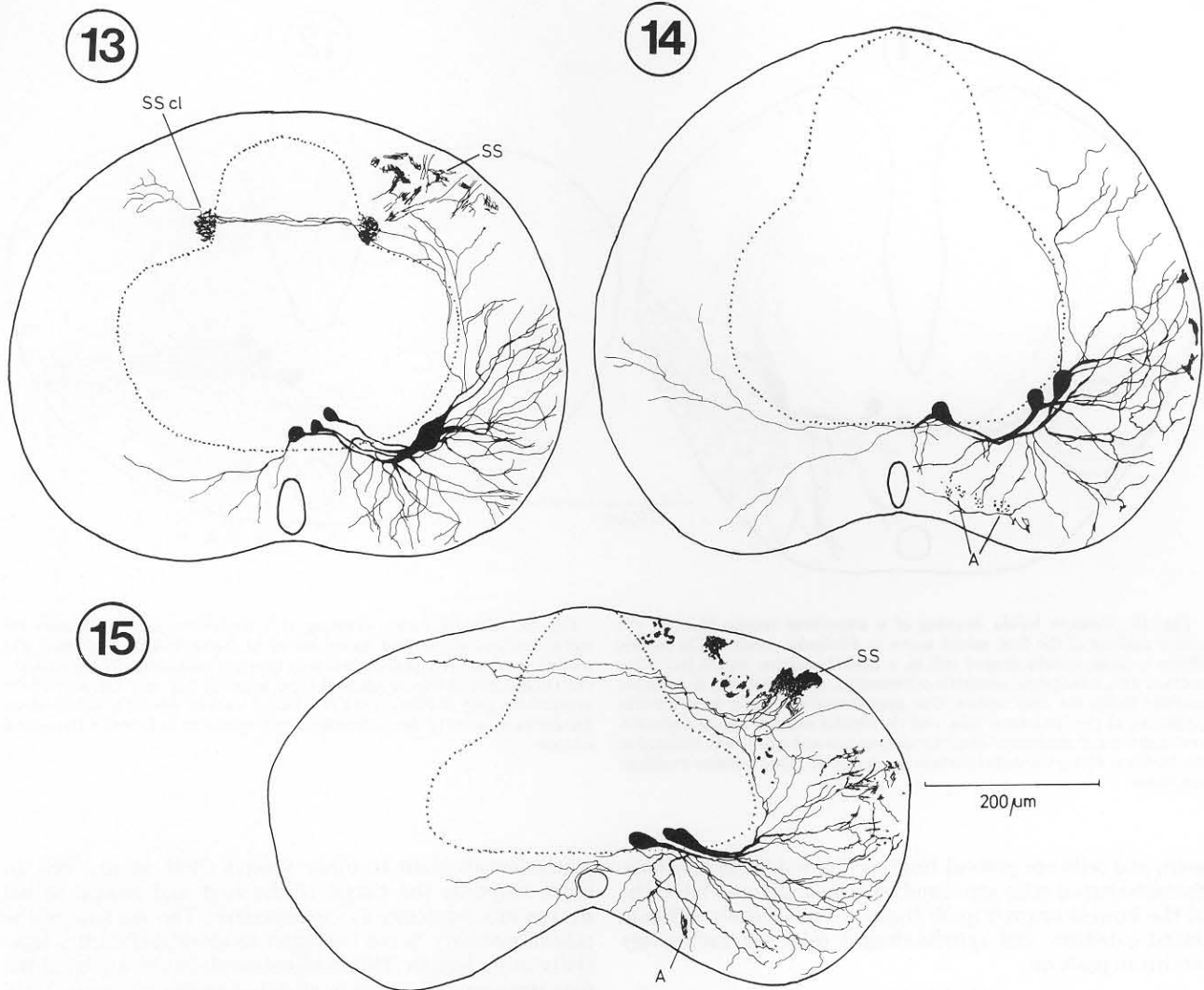


Fig. 13. Camera lucida drawing of a transverse section through the motor nucleus of the second spinal nerve in *Plethodon jordani*. The section shows a large spindle-shaped cell in a ventrolateral position in the white matter. This cell has a wide dendritic arborization that reaches to the dorsal somatosensory fiber tracts. Somatosensory fibers of the second spinal nerve project to the contralateral side. Small pear-shaped cells are situated medially within the gray matter; they generally show narrower dendritic arborization than the spindle-shaped cells. The medialmost cells send dendritic processes to the contralateral side.

Fig. 14. Camera lucida drawing of a transverse section through the motor nucleus of the second spinal nerve in *Rhyacotriton olympicus*. The lateral cells are less differentiated into a spindle-like shape than in Figure

13. These cells lie within the white matter, where they are situated close to the border of the periventricular gray matter. The medialmost cell has extensive contralateral dendritic arborization.

Fig. 15. Camera lucida drawing of a transverse section through the motor nucleus of the second spinal nerve in *Batrachoseps attenuatus*. The section shows spindle-shaped cells situated within the gray matter with extensive dendritic arborization in a pattern that is different from that of the first spinal. Dorsally extending dendrites reach the dorsal somatosensory tracts, which have fibers crossing to the contralateral side. Contralaterally extending dendrites from the medialmost neurons could not be followed to the lateral surface.

nucleus differs in that a clearly distinguishable lateral motor column is lacking. There are medial mostly pear-shaped cells and larger medial to more laterally located polygonal and cone-shaped cells. These latter cells lie inside or slightly outside the periventricular gray matter. Cells of this nucleus are not uniformly aligned; rather, they are dispersed throughout the nucleus. Only in *Salamandra* have we found some spindle-shaped cells in the nucleus of the spinal accessory nerve.

The third class is found in the plethodontid tribe Bolitoglossini as well as in *Rhyacotriton*. Here, the second spinal

and the spinal accessory nuclei (as well as the other spinal nuclei) show a rather unorganized arrangement of pear-shaped and polygonal cells, and no clear distinction exists between a medial and a lateral motor column. This situation resembles cytoarchitecture class 2 but differs in that somata are never found outside the periventricular gray matter. The cytoarchitecture of the first spinal nucleus consists of a rather narrow band of medially situated pear-shaped cells. Whereas the more lateral polygonal cells of the second spinal and spinal accessory nuclei show rather wide arborization, all cells of the first spinal nucleus of the

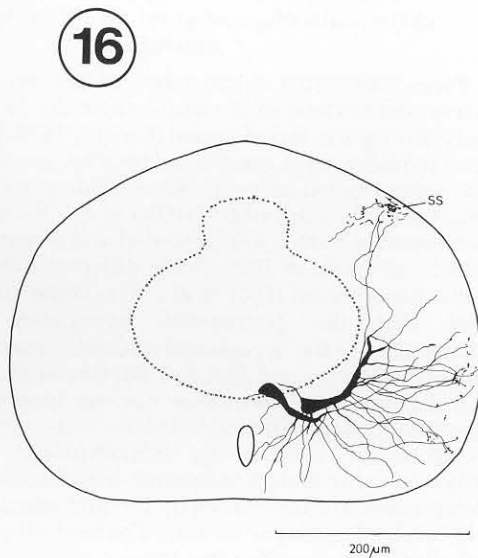


Fig. 16. Camera lucida drawing of a transverse section through the motor nucleus of the accessory spinal nerve in *Eurycea bislineata*. The section shows a cone-shaped neuron situated medially partly within the gray matter. Another cone-shaped cell is found in a more lateral position in the white matter and has more extensive dendritic arborization that reaches the dorsal somatosensory tracts of the second spinal nerve. No crossing dendrites were observed.

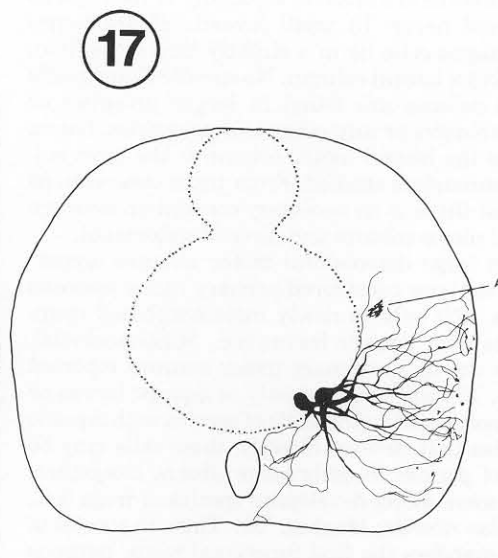


Fig. 17. Camera lucida drawing of a transverse section through the accessory spinal nerve in *Batrachoseps attenuatus*. The section shows one rather spindle-shaped and a more polygonal cell, both situated within the gray matter at the border to the white matter. No crossing dendrites were observed.

Bolitoglossini have a rather narrow, laterally extending dendritic tree.

In anurans (Székely, '76) two types of motor neurons (usually termed secondary motor neurons) are arranged in dorsolateral and ventromedial columns. The former contains relatively large cells with spindle-shaped perikarya. The long axis of these cells is oriented in an oblique ventromedial-dorsolateral direction. The ventromedial column contains relatively smaller cells that are more irregular in shape, with generally pear-shaped perikarya that are more or less horizontal in orientation. These two rows of cells have extensively overlapping dendritic fields.

Oka et al. ('87) report the same "spinal" cytoarchitecture for the spinal accessory nerve in *Bufo japonicus*. With regard to the hypoglossal/second spinal nerve, these authors report that only its ventrolateral nucleus shows a typical spinal cytoarchitecture, whereas the dorsomedial hypoglossal nucleus possesses a cytoarchitecture similar to that of the cranial motor nuclei.

Lateral and medial motor columns also are found in squamates, turtles, and birds, and lateral and medial nuclei are found in mammals (Fetcho, '87). There is a growing awareness that the living amphibians (the Lissamphibia) form a monophyletic group (de Queiroz and Cannatella, '87; Trueb and Cloutier, '87); thus the most parsimonious interpretation of our data is that the first of the three cytoarchitectural classes represents the ancestral pattern for salamanders.

The relatively unordered structure of all spinal motor nuclei of bolitoglossines and of the spinal accessory nucleus in all salamanders except the plethodontid tribe plethodontini is a derived condition that is extraordinary among limbed vertebrates. In limbless lizards as well as in snakes a lateral motor column is absent, although in limbless

lizards the column is present early in ontogeny; in snakes the lateral column never forms (Fetcho, '87).

Ontogenetic considerations

We propose an evolutionary-developmental hypothesis for the cellular arrangement found in bolitoglossines. These salamanders are the most highly derived and specialized lineage of the family Plethodontidae (Larson, '84; Lombard and Wake, '86; Roth and Wake, '85b; Wake, '66; Wake and Larson, '87), and their phylogenesis has been strongly channelled by the cladistically sequential loss of lungs, loss of larval stages, and subsequent developmental evolution, including both paedomorphosis and ontogenetic repatterning (Roth and Wake, '85b; Rettig et al., unpublished data). Present-day bolitoglossines are a mosaic of relatively more juvenile (e.g., their skulls and autopodia) and relatively more adult (e.g., development of ipsilateral retinofugal projections in embryos) characters. We hypothesize that the spinal cord in bolitoglossines represents paedomorphic retention and subsequent elaboration of cytoarchitectural features characteristic of embryos of early larval forms of other salamanders.

In larval and juvenile *A. macrodactylum*, *Eur. bislineata*, *R. olympicus*, and *Salamandra salamandra* (all of which have an aquatic larval stage), the lateral motor column in the nuclei of the first two spinal nerves is not fully differentiated. Only a few large, spindle-shaped neurons are seen, but these are more medial than similar cells in older individuals of these species, which lie in a well-defined lateral motor column. Both the number of spindle-shaped cells and their laterality increase with age (unpublished data). We interpret this as reflecting a process of cell migration.

In the direct-developing plethodontids the situation is more variable. In small juvenile *Plethodon* both lateral and

medial motor columns are evident, especially in the nucleus of the first spinal nerve. In small juvenile *Hydromantes* some spindle-shaped cells lie in a slightly lateral position, but we do not find a lateral column. No spindle-shaped cells and no lateral column are found in larger juveniles or adults of *Hydromantes* or any other bolitoglossines, but in adult *Plethodon* the lateral motor column is the most evident of any salamanders studied. From these observations we conclude that there is no necessary correlation between lack of a lateral motor column and direct development.

We have seen large dorsolateral motor neurons (apparently identical to those considered primary motor neurons by Fetcho, '86a, '87) only in newly metamorphosed members of a species with aquatic larvae (i.e., *Salamandrella*). We predict that the large primary motor neurons reported by Fetcho ('86a, '87) will be found only in aquatic larvae or in newly metamorphosed individuals of species with aquatic larvae. In species that develop directly, these cells may be present for brief periods in embryos or absent altogether, as they are in some direct-developing species of frogs (i.e., *Eleutherodactylus ricordii*; Hughes, '59). Thus, in species of terrestrial salamanders the first functional motor neurons may be the oval or pear-shaped small cells lying medially. Some of these cells gradually migrate laterally during ontogeny to differentiate into spindle-shaped cells and give rise to the lateral motor column, while a substantial proportion of them remain in a rather medial position. Fetcho ('86a) also hypothesized that some of these small medial cells in *Necturus maculosus* are newly born and in the process of migration. In the completely terrestrial and direct-developing bolitoglossines, we believe that the one remaining medial column is composed of cells that are in their definitive position as well as cells that have halted their lateral migration.

Of special interest is the cytoarchitecture of the spinal accessory nucleus: among the species studied we find the full range of different stages of morphological differentiation. In the plethodontid tribe Plethodontini the spinal accessory nucleus is well differentiated. In the plethodontid Hemidactyliini and Desmognathinae, as well as in all nonplethodontid species studied, except *Rhyacotriton*, the spinal accessory nucleus shows no or only very few migrated spindle-shaped cells. Most lateral cells are cone-shaped and may be found inside as well as outside the periventricular gray matter. In all plethodontid Bolitoglossini, as well as in *Rhyacotriton*, the spinal accessory nucleus is as poorly differentiated as the other spinal nuclei; no neurons have migrated into the white matter. Thus, in species other than this latter group, the spinal accessory neurons show a lesser degree of cell migration and differentiation than those of the first and second spinal nuclei. This may be due to a retardation in the development of this nucleus. The cone-shaped cells that mostly occupy an intermediate position and can be found both inside and outside the periventricular gray matter apparently represent an intermediate stage of morphological differentiation between pear-shaped and spindle-shaped cells.

In most major taxa studied the motor nucleus of the first spinal nerve shows a higher degree of cell migration and differentiation than the second spinal and spinal accessory nuclei. Ontogenetic data show that among the cervical spinal motor nuclei the first spinal nucleus differentiates first, followed by the nuclei of the second spinal and then of the spinal accessory nerves.

Comparison with the distribution and cytoarchitecture of cervical spinal nuclei in anurans

Frogs differ from salamanders in lacking a first spinal nerve and nucleus in the adult stage; the nerve is present only during the larval period (Gaupp, 1896–1904). The ramus hypoglossus is constituted by what is considered to be the second spinal nerve. In *Rana* (Matesz and Székely, '77; Stuesser et al., '83) and *Bufo* (Oka et al., '87) this nerve has two separate nuclei, a dorsomedial and a ventrolateral one, which—at least in *Bufo*—have different patterns of dendritic arborization (Oka et al., '87). These differences suggest that the dorsomedial hypoglossal nucleus is homologous to the hypoglossal nucleus of mammals (e.g., of the rat — Matesz and Székely, '83; Oka et al., '87). In *Bufo* the dorsomedial hypoglossal nucleus innervates the hypoglossus, genioglossus, subhyoideus, and—together with the ventrolateral nucleus—the geniohyoideus, all muscles which in salamanders (assuming homologies are correctly determined) are innervated by the first spinal nucleus and are involved in tongue feeding. The ventrolateral hypoglossal nucleus innervates the levator scapulae inferior and superior and the rhomboideus anterior muscles. We have initiated studies to determine the homologies of the hypoglossal nucleus in frogs and salamanders.

Oka et al. ('87) have presented evidence for the existence of a spinal accessory nerve (and nucleus) in *Bufo* that innervates the interscapularis and cucullaris muscles. This nucleus slightly overlaps with the caudal portion of the vagus nucleus at the level of the obex, but its cells are found more ventrally and more medially in relation to the vagus neurons; it extends caudally at least to the caudal end of the second spinal motor neuron column. This is exactly the situation found in salamanders, with the exception that in bolitoglossine salamanders the spinal accessory nucleus starts even more caudally. The neurons of this nucleus were found to have a cytoarchitecture that is distinctly different from that of the cranial motor neurons. Furthermore, neurons of the spinal accessory nucleus do not project to the contralateral side. Both of these conditions characterize the spinal accessory nerve of salamanders as well. We believe that the spinal accessory nucleus in toads is homologous to that found in salamanders. Krammer et al. ('87) have argued that the spinal accessory nerve originated as a mixed nerve of special visceral origin and that during both ontogeny and phylogeny the sensory fibers shift to a spinal pathway, but we have no evidence of a sensory component in adults or during ontogeny in salamanders, and none has been found in anurans either (Oka et al., '87).

Functional considerations

The ventromedial motor column was once thought to innervate axial musculature, and the dorsolateral column was thought mainly to innervate limb musculature (Cruce, '74; Ebbesson, '76; Silver, '42), but recent studies in fishes and amphibians have shown that both axial and appendicular muscles are innervated by both types of cells (Farel and Bemelmans, '80; Fetcho, '87; Stephens and Holder, '85).

An experimental study of the distribution of motor neurons in the axolotl (*A. mexicanum*) showed that poor differentiation of the cells into columns in this neotenic species did not permit clear association of cell structure and location with muscle function (Székely and Czeh, '67). A recent

study of innervation of the limb musculature in the same species found two types of motor neurons in the cord (Stephens and Holder, '85). The majority were relatively large, spindle-shaped cells lying in the ventral horn, while the second type were smaller, more medial, and also ventral, in position. The motor neuron pool for each limb muscle studied included both types of cells. Some separation (both rostrocaudal and mediolateral) of the motor neurons with axons in dorsal and ventral rami of spinal nerves is seen in *Necturus* (Fetcho, '86a), but the locations of the subpopulations overlap substantially. There is accordingly much less topographic organization and spatial segregation in the spinal cord of *N. maculosus* and in *A. mexicanum* than in amniotes (Fetcho, '86a,b, '87; Stephens and Holder, '85) and possibly less than in some teleosts (Fetcho, '86a) and elasmobranchs (Droge and Leonard, '83). Whether this will be found to be the result of the permanently larval state of these amphibian species will depend on further experimental work.

Motor pools in amniotes are spatially segregated in the cord, with those innervating epaxial musculature being located ventromedially and those innervating hypaxial musculature being dorsolateral in position (Fetcho, '86b, '87). Because of these facts it has been argued that a substantial reorganization of the motor columns must have been necessary to account for the differences between amniotes and other vertebrates (Fetcho, '86a, '87). However, our results show that in fully metamorphosed and adult salamanders (with the exception of bolitoglossines) motor columns with distinctly different kinds of cells are present, and studies of these species should be conducted before concluding that there are major differences between amphibians and amniotes.

Lateral motor neurons in goldfish innervate fast-contracting white muscle fibers and smaller medial motor neurons innervate slow-contracting red muscle fibers (Fetcho, '86a). If these results with goldfish also apply to metamorphosed salamanders, one might expect that pear-shaped cells innervate slow-contracting muscle fibers in all salamanders, and the polygonal cells of bolitoglossines, like the spindle-shaped cells in other salamanders, should innervate twitch fibers. While red and white fibers are present in the axial musculature of frogs (Forrester and Schmidt, '70) and salamanders (Lee, '71; Watanabe et al., '80; Roth et al., unpublished data), the relationship between neuron type and muscle fiber type remains unknown in all amphibians (Fetcho, '87).

Bolitoglossine salamanders differ from most plethodontids in locomotor behavior in that they move slowly and do not have well-developed escape behavior; frequently their only antipredatory behavior involves immobility (Dodd and Brodie, '76). We have photographic evidence that bolitoglossines differ markedly from other salamanders in their feeding behavior. Typically terrestrial salamanders combine a tongue thrust with a forward lunge, which results from rotation of the limbs on the girdles. Bolitoglossines have remarkable abilities to project the tongue at high speed and for great distance. In contrast to other salamanders, the head usually is held in place caudorostrally, and there is no forward lunge. The anatomical reorganization that has taken place in the bolitoglossines suggests that study of the characteristics of their apparently paedomorphic spinal cord may clarify the functional implications of cellular differentiation and migration in the spinal cord of vertebrates in

general. While we have identified a correlation between behavior and neuronanatomical details (see also Roth and Wake, '85a), a mechanistic interpretation remains a challenge for future research.

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