

Motor Neurons and Motor Columns of the Anterior Spinal Cord of Salamanders: Posthatching Development and Phylogenetic Distribution

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Key Words. Amphibians · Cervical spinal cord · Primary motoneurons · Secondary motoneurons · Motor columns, lateral and medial

Abstract. The posthatching development of rostral (1st–4th) spinal motor neurons was studied in ten species of salamanders, using horseradish peroxidase and cobaltic lysine tracing techniques. Development of spinal motor neurons differs among species in association with differences in life history and general developmental patterns (i.e., between species with aquatic larvae versus those with direct development, with or without ontogenetic repatterning). In the plesiomorphic state, represented by species with aquatic larvae, five types of motor neurons are present: (1) large, multipolar neurons, believed to be primary motor neurons; (2) medial, pear-shaped neurons; (3) larger, spindle-shaped neurons, which increase in number during posthatching development; (4) cone-shaped neurons, and (5) bilaterally arborizing neurons (found only at the rostral pole of the first spinal nucleus). Direct-developing desmognathine salamanders have the plesiomorphic set of motor neurons, but appear to lack Mauthner neurons. Direct-developing plethodontine salamanders have cone-shaped, pear-shaped, and spindle-shaped neurons, but lack primary motor neurons and Mauthner neurons. Direct-developing bolitoglossine salamanders, which exhibit both pedomorphosis and ontogenetic repatterning, have only medial, pear-shaped neurons, and lack primary motor neurons, spindle-shaped neurons, cone-shaped and bilaterally arborizing neurons. At all developmental stages in all species studied, pear-shaped neurons are always found in medial positions and spindle-shaped neurons are always found in lateral positions. Spindle-shaped neurons are found more laterally as development proceeds. The medial and lateral motor columns of salamanders and amniotes differ in their connections with peripheral targets (i.e., axial muscles vs. limbs). This implies a lack of homology of neuron types in salamanders and amniotes, which has been obscured by the current terminology.

Introduction

The morphology and development of spinal motor neurons have been studied extensively in amphibians [Silver, 1942; Hughes, 1959; Székely, 1976; Stephens and Holder, 1985; Roth and Wake, 1985a; van Mier et al., 1985; Fetcho, 1986a; Nordlander, 1986; Roth et al., 1988; Wake et al., 1988]. Motor neurons of amphibians, like those of other vertebrates, have been classified based on: (1) time of appearance (i.e., primary vs. secondary); (2) position (i.e., medial vs. lateral); (3) morphology (i.e., multipolar vs. bipolar vs. unipolar),

and (4) connections (i.e., limb vs. axial muscle targets).

In most amphibians and other anamniotes, the earliest motor neurons to appear during development, termed primary motor neurons, are large and multipolar, with several thick main dendrites that originate from the soma [Youngstrom, 1940]. They have been shown to receive direct monosynaptic input from Mauthner neurons and to innervate axial muscles in teleost fishes [Fetcho and Faber, 1988], in which they appear to function in Mauthner-mediated escape behavior. In teleosts, primary motor neurons also play a

role in high speed escape behavior. In teleosts, primary motor neurons are large and multipolar, with several thick main dendrites that originate from the soma [Youngstrom, 1940]. They have been shown to receive direct monosynaptic input from Mauthner neurons and to innervate axial muscles in teleost fishes [Fetcho and Faber, 1988], in which they appear to function in Mauthner-mediated escape behavior. In teleosts, primary motor neurons also play a

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There are several types of motor neurons in the spinal cord. The primary motor neurons are large and multipolar, with several thick main dendrites that originate from the soma [Youngstrom, 1940]. They have been shown to receive direct monosynaptic input from Mauthner neurons and to innervate axial muscles in teleost fishes [Fetcho and Faber, 1988], in which they appear to function in Mauthner-mediated escape behavior. In teleosts, primary motor neurons also play a

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A second type of motor neuron is the secondary motor neuron. These are smaller and more bipolar or unipolar. They are found in lateral positions and are thought to innervate limb muscles. In teleosts, secondary motor neurons also play a

role in high-speed swimming behavior [Liu and Westfield, 1988]. Synapses between Mauthner neurons and primary motor neurons have been inferred from intracellular recordings in frogs [Lee, 1982]. They have yet to be demonstrated in salamanders, but the axons of primary motor neurons travel in close proximity to the Mauthner axon, and contacts are assumed to occur [Fetcho, 1986a, 1987].

The next motor neurons to appear during development, termed secondary motor neurons [Youngstrom, 1940], are of two types: small, pear-shaped neurons, situated in a medial position close to the central canal, and larger spindle-shaped neurons, that lie at or just outside the ventrolateral border of the periventricular gray matter. In most amphibians, pear-shaped neurons constitute a medial motor column and spindle-shaped neurons constitute a lateral motor column [Székely, 1976; Wake et al., 1988].

There is confusion concerning the different morphological and functional types of motor neurons that appear during amphibian development [Fetcho, 1987]. One source of confusion stems from terminology based on the time of appearance of motor neurons during development (i.e., primary and secondary motor neurons). This terminology requires the implicit assumption that a common pattern of spinal cord development occurs in all amphibian species. It is often assumed that all anamniotes possess primary motor neurons. However, some species, such as *Eleutherodactylus ricordii* [Hughes, 1959], have a modified program of motor neuron development involving the loss of primary motor neurons. Differences among amphibian species in the time of appearance of motor neurons during development is a potential source of terminological confusion, because types of motor neurons that have been named for their time of appearance during development (i.e., 'primary' and 'secondary' motor neurons) may in fact differ in their time of appearance among different species. Furthermore, neurons appearing at the same relative times may differ in their morphology or connections.

A second source of confusion stems from terminology based on the position of neurons within the spinal cord (i.e., medial and lateral motor columns). In amniotes, the medial motor column innervates axial musculature and the lateral motor column innervates limb musculature [Fetcho, 1986b, 1987]. Anatomically speaking, salamanders also possess medial and lateral motor columns [Wake et al., 1988]. However, several studies have shown not only that limb muscles are in-

nervated by neurons of both the medial and the lateral motor columns [Stephens and Holder, 1985], but also that axial muscles are innervated by both types of neurons [Fetcho, 1986a; Roth and Wake, 1985a; Wake et al., 1988; Nishikawa, unpubl. observations]. These problems are deeper than mere terminology, however, because of the implicit assumption that neuron types with the same names (e.g., primary motor neurons or medial motor columns) are homologous in the different taxa that possess them.

The present study investigates the posthatching development of motor neurons and motor columns of the four anteriormost spinal nerves in ten species of salamanders. Studies of early embryonic development were not feasible in some of the taxa we studied (i.e., *Plethodon* and *Hydromantes*), because they are difficult to breed in captivity and, in nature, their eggs are laid in underground caves. The first and second spinal nerves innervate muscles involved in aiming (hypobranchial muscles) and retracting the tongue (hypaxial muscles) during feeding, as well as epaxial neck muscles [Roth and Wake, 1985a]. The third and fourth spinal nerves also innervate the hypaxial tongue retractor muscles, as well as muscles of the pectoral girdle and forelimb [Francis, 1934]. We compare the posthatching development of motor neurons among different spinal segments, and among salamander species that differ in life history and development strategies. Of special importance are comparisons between species with an aquatic larval stage and species with different patterns of direct development.

We also compare the types of spinal motor neurons found in salamanders with those of frogs and amniotes. In amniotes, there is a good correspondence among the time of appearance, morphology, position and connections of motor neurons (e.g., lateral, spindle-shaped motorneurons innervate limb muscles). We show here that the attributes of motor neurons do not correspond with each other in salamanders as they do in amniotes. This lack of correspondence implies a lack of homology of neuron types between salamanders and amniotes, which has been obscured by the current terminology.

Material and Methods

The original research reported herein was performed under the guidelines established by the National Science Foundation and the National Institutes of Health for the use of vertebrate animals in research. Salamanders from the two largest urodele fam-

Table 1. Size classes (mm) of each species examined in this study

Species	Hatchlings	Early larvae	Mid larvae	Late larvae	Juveniles	Adults
<i>P. waltl</i>	–	5.7–6.4 (4)	7.7–10.2 (2)	18.1–34.5 (3)	–	–
<i>S. salamandra</i>	10.0–19.0 (2)	19.5–21.0 (2)	20.0–22.4 (2)	25.0–30.0 (1)	–	–
<i>E. bislineata</i>	10.6 (1)	10.5–15.9 (4)	15.9–19.9 (4)	20.0–24.9 (7)	26.0 (1)	≥38.5 (2)
<i>P. ruber</i>	–	13.5–19.9 (2)	–	30.0–38.0 (5)	–	–
<i>D. quadramaculatus</i>	–	–	29.5 (1)	39.0–39.5 (2)	–	–
<i>D. monticola</i>	–	–	14.5–16.0 (5)	–	–	–
<i>D. ochrophaeus</i>	–	–	10.0–14.5 (11)	–	–	≥43.9 (4)
<i>D. aeneus</i>	13.2 (1)	–	–	–	15.2–20.0 (2)	≥23.6 (4)
<i>P. jordani</i>	–	–	–	–	17.5–30.0 (4)	≥34.8 (2)
<i>H. italicus</i>	–	–	–	–	20.0–37.0 (9)	≥42.0 (4)

Sample sizes are given in parentheses. Hatchlings have not yet fully resorbed their yolk sacs. Ages are available only for *S. salamandra* and *P. waltl* reared in captivity. For *Salamandra*, hatchlings are 2–5 days posthatching, early larvae are 1 week posthatching, mid larvae are 3 weeks posthatching and late larvae are 7 weeks posthatching. For *Pleurodeles*, hatchlings are 7 days posthatching, early larvae are 14 days posthatching, mid larvae are 4–8 weeks posthatching, and late larvae are 16–40 weeks posthatching.

ilies, Salamandridae (*Salamandra salamandra* and *Pleurodeles waltl*) and Plethodontidae, were studied. Numbers of specimens of each species are given in table 1. The two salamandrid species have aquatic larvae. However, adult *Salamandra* are terrestrial and adult *Pleurodeles* are aquatic. We studied all four major groups of the family Plethodontidae: subfamily Desmognathinae (*Desmognathus quadramaculatus*, *Desmognathus monticola*, *Desmognathus ochrophaeus*, and *Desmognathus aeneus*); subfamily Plethodontinae, tribe Hemidactyliini (*Eurycea bislineata* and *Pseudotriton ruber*), tribe Plethodontini (*Plethodon jordani*), and tribe Bolitoglossini (*Hydromantes italicus*).

The desmognathine species, *D. quadramaculatus*, *D. monticola* and *D. ochrophaeus* possess aquatic larvae, whereas *D. aeneus* shows direct development and lacks an aquatic larval stage (i.e., eggs are laid on land and fully transformed individuals hatch from the eggs). Adult *D. quadramaculatus*, *D. monticola* and *D. ochrophaeus* are aquatic to semiaquatic, whereas adult *D. aeneus* are terrestrial. The two hemidactyliine species, *E. bislineata* and *P. ruber*, have aquatic larvae and are aquatic to semiaquatic as adults. The plethodontine *P. jordani* and the bolitoglossine *H. italicus* are direct developers with no aquatic larval stage, and are terrestrial throughout life, as are all plethodontine and bolitoglossine salamanders.

D. quadramaculatus, *D. monticola*, *D. ochrophaeus*, *P. ruber*, and *P. jordani* were collected near the Highlands Biological Station, Highlands, N.C., and *D. aeneus* were collected at War Woman Dell, Rabun County, Ga. *H. italicus* were collected in the Apuan Alps of the Italian Apennines between the cities of Lucca and La Spezia. Adult *S. salamandra* and *P. waltl* were obtained from commercial dealers. Their larvae came from breeding colonies of the BRI Bremen. Experiments were carried out at the Highlands Biological Station and the BRI Bremen in 1988 and 1989.

Because the true age of animals captured in the wild cannot be estimated accurately, we staged the animals according to their snout-vent length (SVL). Exact age data were available only for larvae of *Salamandra* and *Pleurodeles* born in the laboratory. How-

ever, the presence of yolk in some wild-caught specimens indicates that they had hatched within a week of capture. For each species, the SVLs of each size class are given in table 1.

Motor neurons of the first through fourth spinal nerves were stained retrogradely by application of either horseradish peroxidase (HRP) or cobaltic lysine to transected nerve stumps close to the brain. In all specimens, the first and second spinal nuclei, and sometimes the third and fourth spinal nuclei, were stained.

Methods for the application of HRP follow Wake et al. [1988]. Animals were anesthetized in 1.0% tricaine methanesulfonate (MS 222) for 1–5 min, depending on the species. The first through fourth spinal nerves were exposed near the brain, and crystalline HRP (Boehringer grade I) was applied to the cut ends of transected nerves. After 2- to 3-day survival period, the animals were sacrificed by overanesthesia in 1.0% MS 222 for 15–20 min. The HRP was reacted with diaminobenzidine in whole mounts or in frozen sections, following standard procedures [Roth and Wake, 1985a]. Whole mounts were subsequently cleared in methyl salicylate, and some were returned to alcohol, embedded in Epon, and cut in transverse sections at 30 μ m.

For cobaltic lysine experiments [Göres et al., 1979; Matsushima et al., 1988], animals were anesthetized by immersion in 1.0% MS 222, followed by immobilization with Succinyl-AstaTM. The cut nerve stump was placed into a small glass tube (diameter 0.2–0.5 mm) filled with cobaltic lysine. The tube was sealed using a mixture of paraffin and vaseline. The preparation was placed in a refrigerator at 4°C for 24–36 h, after which the animal was sacrificed by overanesthesia in 1.0% MS 222 as above. The brain was removed from the skull and placed in 0.1 M phosphate buffer saturated with H₂S for 10 min, after which it was dehydrated, embedded in paraffin and cut into 30- μ m transverse sections. The sections were intensified with Gallyas sodium tungstate developer and counterstained with nuclear fast red.

Serial cross-sections of the brainstem were examined for the presence or absence of Mauthner neurons. Absence of neurons with very large somata in the vicinity of the entrance of the eighth cranial nerve was assumed to indicate that Mauthner neurons

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were absent. It is possible, however, that these neurons were present, but in a much reduced form.

Cell types and their locations were determined in HRP whole mounts using a dissecting microscope. The neurons were mapped along the mediolateral axis. Each side of the spinal cord was divided into ten longitudinal positions, with position 1 medialmost, situated next to the central canal, and position 10 lateralmost, situated next to the subpial surface. For each stained neuron, its morphological type and its mediolateral location were recorded. In all of the specimens studied, the border between the gray and the white matter of the spinal cord lies at position 5 at the dorsoventral level where the gray matter is widest. At more ventral levels, where the gray matter is narrower because of its oval shape, the border lies at position 4.

Data on neuron numbers and positions were analyzed using StatviewTM 512+ for the MacIntosh Plus computer. The Pearson product-moment correlation between SVL and the proportion of spindle-shaped neurons in the spinal cord was tested for significance at the $\alpha = 0.05$ level.

Results

Staining Success

In most cases, retrograde HRP labeling more or less completely stained the motor neurons, including fine dendrites, in both whole mounts and sections. The best results were obtained from brains reacted as whole mounts, embedded in Epon, and cut in serial sections. The quality of tissue preservation was lower in brains that were sectioned before reaction with diaminobenzidine. Cobaltic lysine generally resulted in more complete staining of neurons than labeling with HRP. However, this method has other limitations. Cobaltic lysine can only be used for staining sections and not whole mounts. However, it is much easier to count neurons in whole mount preparations. Also, cobaltic lysine can only be used on relatively large nerve stumps and, therefore, cannot be used for small hatchlings and larvae.

Types of Spinal Motor Neurons

Primary Motor Neurons (fig. 1). Primary motor neurons are found both medially and laterally within the spinal cord, at positions 2–5. Their somata are most often situated at the border between the gray and white matter, and have a cap-like shape. Two thick primary dendrites extend along the margin of the gray matter in medial and dorsolateral directions. Three to four somewhat smaller dendrites originate from the primary dendrites and from the soma. These arborize into many fine dendrites which fill most of the ventral to dorsolateral white matter. Medial dendrites do not

cross the midline, except for a very few short dendrites which cross to the contralateral side in the vicinity of the central canal.

We did not attempt to observe a close association between primary motor neurons and Mauthner axons in our material, nor did we determine that these neurons are the first to appear during development. Primary motor neurons were distinguished from other types of neurons on the basis of morphology alone. In most species, primary motor neurons could be reliably distinguishable from other motor neurons because they had the largest somata, the largest axon diameters, and were the most strongly multipolar, with the most extensive ipsilateral arborization of any neuron type. In *Eurycea*, these differences were less distinct than in the other species. Unlike Fetcho [1986a], we did not observe any segregation of the axons of primary motor neurons in the ventral root in any species. While the primary motor neurons described here resemble those described for other amphibian species [Fetcho, 1986a; Nordlander, 1986; Forehand and Farel, 1982], we cannot be certain that these are the first neurons to develop in the taxa we studied.

Pear-Shaped Neurons (fig. 2). These motor neurons are always found ventromedially, at positions 1–3. They have small, pear-like somata with one thick dendrite that extends laterally to dorsolaterally into the white matter. The secondary dendrites arborize mostly in ventrolateral, lateral and dorsolateral directions. Smaller medial dendrites may be found extending laterally into the contralateral spinal cord and often reach the contralateral subpial zone.

Spindle-Shaped Neurons (fig. 3). These motor neurons are found at mediolateral positions 3–5, at the border of, or slightly outside the gray matter. In most cases, their somata are larger than those of pear-shaped neurons. Two thick primary dendrites originate from the soma and extend in dorsolateral and ventromedial directions, where they arborize extensively. Smaller dendrites also arise from the soma. Medial dendrites terminate at or near the midline of the spinal cord, and have not been observed to cross to the contralateral side.

Cone-Shaped Neurons (fig. 4). These motor neurons are found both medially and laterally, at positions 1–5, both inside and outside the gray matter. They have a very thick, cone-shaped primary dendrite from which relatively thin secondary dendrites originate. These arborize mostly in lateral and ventrolat-

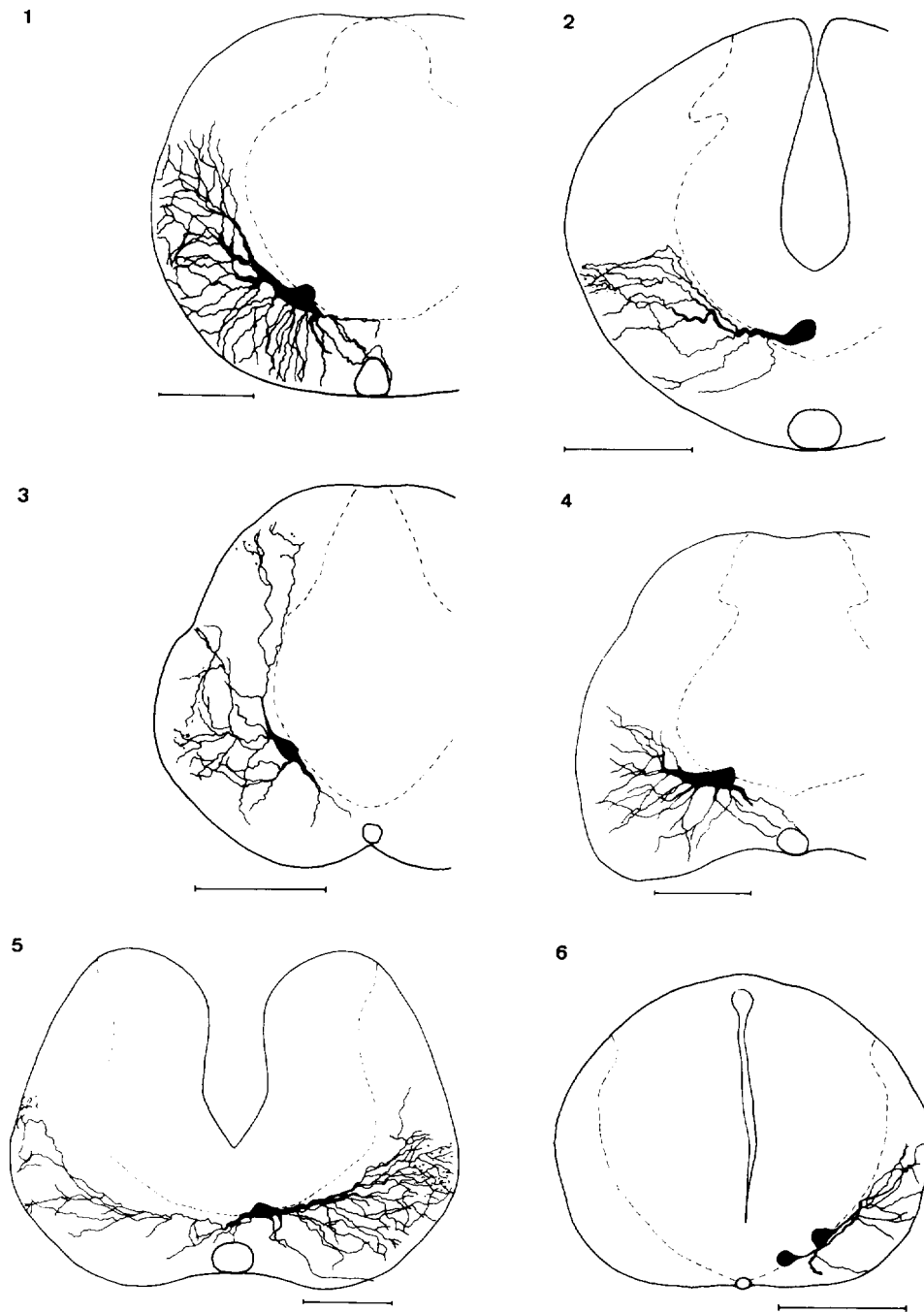


Fig. 1. Camera lucida drawing of a cross-section of the spinal cord of a 7-day-old *S. salamandra* larva (19.5 mm SVL) showing a primary motor neuron. Bar = 100 μ m.

Fig. 2. Camera lucida drawing of a cross-section of the spinal cord of an *E. bislineata* larva (10.5 mm SVL) showing a pear-shaped motor neuron. Bar = 100 μ m.

Fig. 3. Camera lucida drawing of a cross-section of the spinal cord of a *D. ochrophaeus* larva (10 mm SVL) showing a spindle-shaped motor neuron. Bar = 100 μ m.

Fig. 4. Camera lucida drawing of a cross-section of the spinal cord of an *E. bislineata* larva (24.2 mm SVL) showing a cone-shaped motor neuron. Bar = 100 μ m.

Fig. 5. Camera lucida drawing of a cross-section of the spinal cord of a *P. ruber* larva (13.5 mm SVL) showing a bilaterally projecting motor neuron in the rostral part of the first spinal motor nucleus. Bar = 100 μ m.

Fig. 6. Camera lucida drawing of a cross-section of the spinal cord of a 7-day-old *P. waltl* larva (5.7 mm SVL) showing a pear-shaped and a primary motor neuron. Bar = 100 μ m.

Fig. 7. Camera lucida drawing of a cross-section of the spinal cord of a 7-day-old *S. salamandra* larva (19.5 mm SVL) showing a primary motor neuron with HRP. Bar = 100 μ m.

Fig. 8. Camera lucida drawing of a cross-section of the spinal cord of a 7-day-old *S. salamandra* larva (19.5 mm SVL) showing a primary motor neuron with HRP. Bar = 200 μ m.

Fig. 9. Camera lucida drawing of a cross-section of the spinal cord of a 7-day-old *S. salamandra* larva (19.5 mm SVL) showing a primary motor neuron with HRP. Bar = 200 μ m.

Fig. 10. Camera lucida drawing of a cross-section of the spinal cord of a 7-day-old *S. salamandra* larva (11.5 mm SVL) showing a primary motor neuron with HRP. Bar = 200 μ m.

eral direction medially. Bilaterally projecting neurons are found in the spinal nuclei of salamanders, and their position, class (fig. 7-13). T

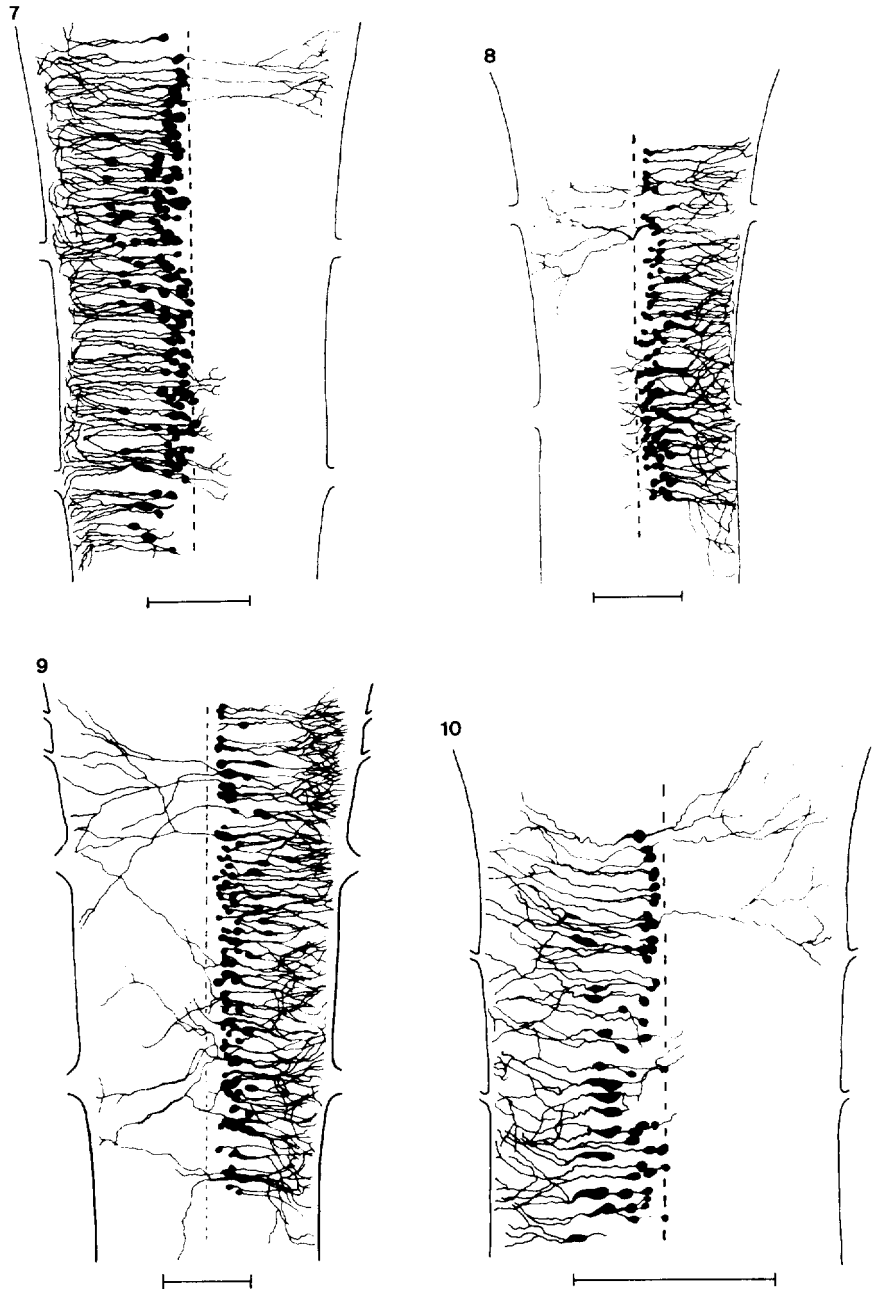


Fig. 7. Camera lucida drawing of a whole mount of the spinal cord of a 21-day-old *S. salamandra* larva (20.0 mm SVL) showing the medial and lateral motor columns stained with HRP. Bar = 200 μ m.

Fig. 8. Camera lucida drawing of a whole mount of the spinal cord of an *E. bislineata* larva (23 mm SVL) showing the medial and lateral motor columns stained with HRP. Bar = 200 μ m.

Fig. 9. Camera lucida drawing of a whole mount of the spinal cord of a *P. ruber* larva (36.4 mm SVL) showing the medial and lateral motor columns stained with HRP. Bar = 200 μ m.

Fig. 10. Camera lucida drawing of a whole mount of the spinal cord of a *D. ochrophaeus* larva (11.5 mm SVL) showing the medial and lateral motor columns stained with HRP. Bar = 200 μ m.

er directions, except for a few dendrites that arborize medially near the central canal.

Bilaterally Arborizing Neurons (fig. 5). These neurons are found only in the anterior half of the first spinal nucleus of all species except bolitoglossine salamanders, and are always situated in a very medial position, close to the midline of the spinal cord (fig. 7-13). They are strongly bipolar, and send lateral-

ly oriented dendrites to both sides of the spinal cord. Dendrites reach both subpial surfaces. Most of the medial, pear-shaped neurons also have contralaterally projecting dendrites, but these are always considerably weaker than the ipsilaterally projecting dendrites. Also, most of the somata of medial, pear-shaped neurons are not located exactly at the midline of the spinal cord.

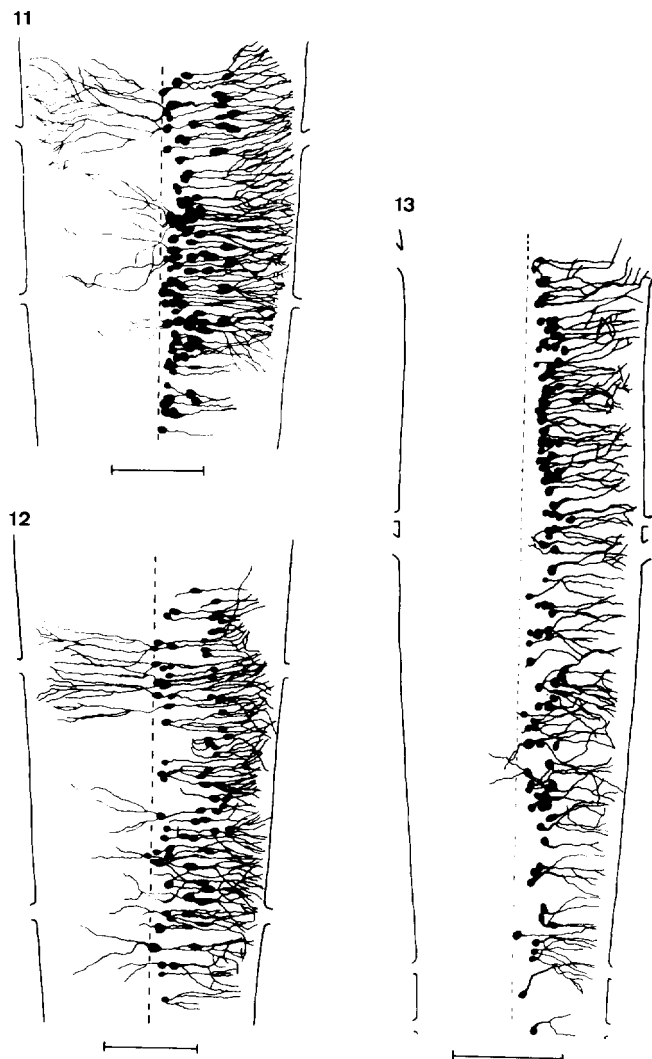


Fig. 11. Camera lucida drawing of a whole mount of the spinal cord of a *D. aeneus* hatchling (13.2 mm SVL) showing the well-developed lateral motor column stained with HRP. Bar = 200 μ m.

Fig. 12. Camera lucida drawing of a whole mount of the spinal cord of a *P. jordani* juvenile (17.5 mm SVL) about 1 year after hatching, showing the medial and lateral motor columns stained with HRP. Bar = 200 μ m.

Fig. 13. Camera lucida drawing of a whole mount of the spinal cord of a *H. italicus* juvenile showing the absence of a lateral motor column and the relative disorganization of somata and dendrites of spinal motor neurons compared to adults of nonbolitoglossine species. Bar = 200 μ m.

Development and Distribution of Neuron Types among Species

P. waltl (fig. 6). The smallest larvae studied ($n = 2$, 7 days after hatching, 5.7 and 6.0 mm SVL) possessed well-developed medial and lateral motor columns,

constituted by pear-shaped and spindle-shaped neurons, respectively. Pear-shaped neurons were found in positions 1–3, with a concentration in position 2. Spindle-shaped neurons were found in positions 3–5, with a concentration in position 4. In a 4-month-old larva (18.4 mm SVL), one pear-shaped neuron was found in position 2. Primary motor neurons were found at positions 3–5 in individuals of all ages (fig. 6). In general, these cell types were more difficult to distinguish in *Pleurodeles* than in other species because there were many neurons of intermediate shape.

S. salamandra (fig. 7). At the earliest age studied ($n = 1$, 1–2 days after hatching, 10.4 mm SVL), two motor columns were clearly present, although the proportion of spindle-shaped neurons was lower than in older larvae (fig. 14). Pear-shaped neurons were found in positions 1–3, with a concentration in position 2, whereas spindle-shaped neurons were found in positions 3–5 with the majority in position 4 (fig. 15a). The ratio of pear-shaped to spindle-shaped neurons was 3.9:1 in the first, 5.1:1 in the second and 6.4:1 in the third spinal nucleus. Primary motor neurons were found in positions 1–4 in the first and second spinal nuclei. Numerous cone-shaped neurons were found at positions 2 and 3 in the first spinal nucleus, and at position 3 in the second spinal nucleus. Up to three bilaterally arborizing neurons were found at the rostral end of the first spinal nucleus (fig. 7).

In *Salamandra*, the percentage of spindle-shaped neurons increases during larval development. In 1- to 2-day-old larvae, only 25% of stained neurons are spindle-shaped. By 7 days after hatching, the percentage of spindle-shaped neurons has increased to about 45%. In *Pleurodeles* larvae, about 40% of stained neurons are spindle-shaped by 7 days after hatching. Thus, in 7 days posthatching salamandrids, the percentage of spindle-shaped neurons is close to the adult value of 50% (fig. 14). Spindle-shaped neurons are found more laterally, on average, at later developmental stages. In hatchlings, spindle-shaped neurons tend to be most abundant at positions 3 and 4, whereas in adults they are most abundant in positions 4 and 5. Thus, the motor columns become more distinct at later developmental stages.

E. bislineata (fig. 8). The smallest larva of this species (10.5 mm SVL), which had already completely resorbed its yolk, had only a few spindle-shaped neurons in the lateral motor column (10 of 75 stained neurons). A slightly larger larva (10.6 mm SVL), which still possessed its yolk sac and thus was recently

hatched, and no lateral motor column was present. Almost all neurons were found in positions 1–3. Only 8% of neurons were pear-shaped neurons.

Fig. 14. Percentage of pear-shaped and spindle-shaped neurons in the lateral motor column in *S. salamandra* increases from hatching. Adult values are shown for comparison.

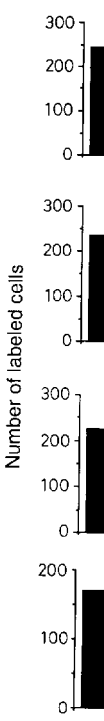


Fig. 15. Bar graphs showing the number of labeled cells in the spinal cord of *Salamandra* and *Pleurodeles* at different developmental stages: *S. salamandra* (a, b, c) and *P. jordani* (d).

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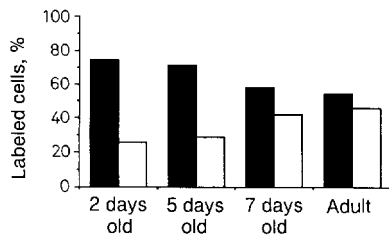


Fig. 14. Proportion of pear-shaped (■) and spindle-shaped (□) neurons in the lateral motor column of the first spinal motor nucleus in *S. salamandra*. The proportion of spindle-shaped neurons increases from about 25% at hatching to about 45% by 7 days after hatching. Adult *Salamandra* have about 50% spindle-shaped motor neurons in the first spinal motor nucleus.

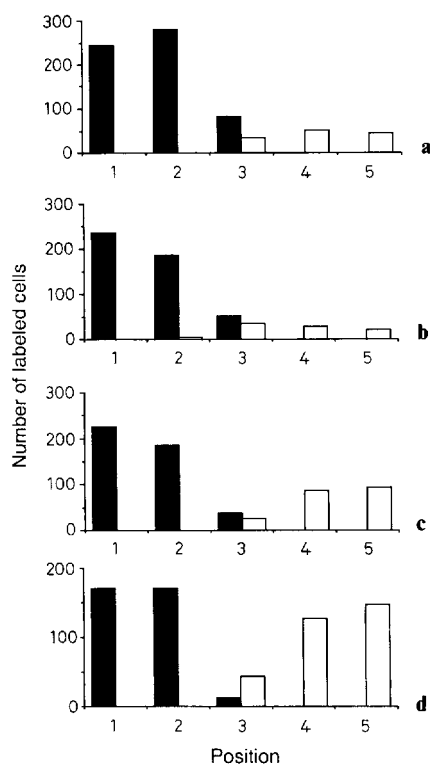


Fig. 15. Bar graphs showing the positions of pear-shaped (■) and spindle-shaped (□) neurons in larvae of four species of salamanders: *S. salamandra* (a), *P. ruber* (b), *D. aeneus* (c) and *P. jordani* (d).

hatched, and a much larger larva (23 mm SVL), had no lateral motor column at all.

Almost all pear-shaped neurons occur in positions 1-3. Only 8 of 747 stained pear-shaped neurons were found in position 4 (fig. 16a). The majority of pear-shaped neurons in all four spinal nuclei were found in

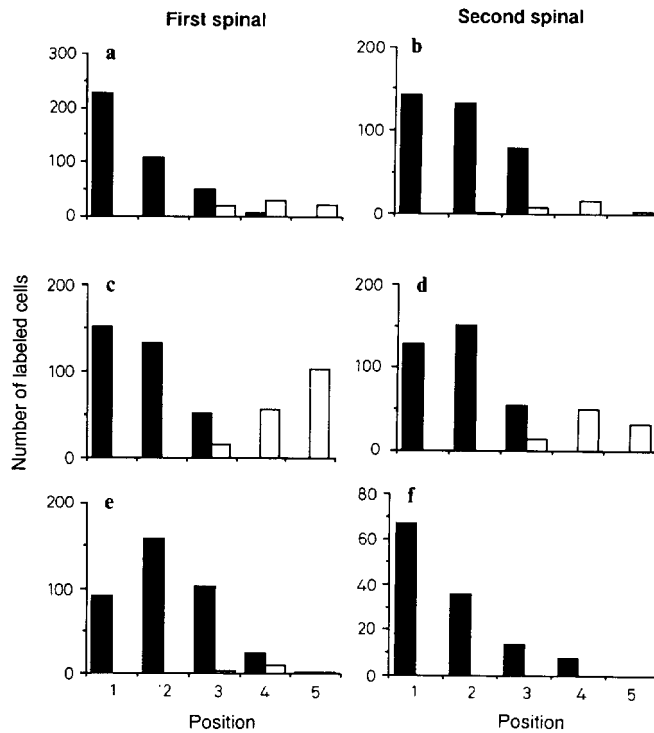


Fig. 16. Bar graphs showing the positions of pear-shaped (■) and spindle-shaped (□) neurons in the first (a, c, e) and second (b, d, f) spinal motor nuclei of larval salamanders: *E. bislineata* (a, b), *D. ochrophaeus* (c, d), and *H. italicus* (e, f). All stained neurons in all individuals are included. Note that the motor columns overlap less in the first than in the second spinal motor nucleus.

position 1. In contrast, almost all spindle-shaped neurons were found in positions 3-5, with the exception of a single neuron which was found in position 2 (fig. 16b). The majority of spindle-shaped neurons were found in position 4. The ratio of pear-shaped to spindle-shaped neurons was 6:1 in the first, 9.3:1 in the second, and 4.5:1 in the third spinal nucleus. Cone-shaped neurons were present in positions 2 and 3 in all four anterior spinal nuclei. Primary motor neurons were found in positions 1-5 in all four nuclei of all larval stages, but were not found in juveniles or adults. Two to eight bilaterally arborizing neurons were found in all larvae at the rostral end of the first spinal nucleus (fig. 8).

As in *Salamandra*, the relative number of spindle-shaped neurons increased significantly with SVL in *Eurycea* (fig. 17). However, it appears that the development of the lateral motor column occurs later in *Eurycea* than in *Salamandra*. In hatchling *Salamandra*,

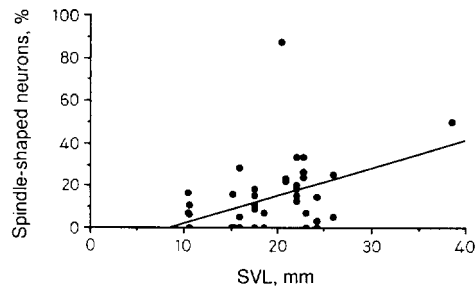


Fig. 17. Relationship between SVL and proportion of spindle-shaped motor neurons in *E. bislineata* ($n = 42$ larvae, $r = 0.42$, $p < 0.01$).

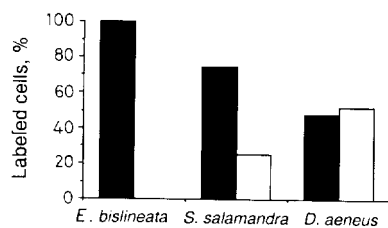


Fig. 18. Proportion of pear-shaped (■) and spindle-shaped (□) motor neurons in the first spinal motor nucleus of hatchlings of *E. bislineata*, *S. salamandra* and *D. aeneus*.

about 25% of stained neurons are spindle-shaped, compared to 0% in hatchlings of *Eurycea* (fig. 18). Also, as in *Salamandra*, the spindle-shaped neurons are found more laterally at later developmental stages.

P. ruber (fig. 9). Medial and lateral motor columns were well developed in the smallest larva studied (13.5 mm SVL), which was not recently hatched. Pear-shaped neurons were found only in positions 1–3, with a concentration in position 1. Spindle-shaped neurons were found in positions 3–5, with the exception of 4 of 90 neurons which were found in position 3 (fig. 15b). The ratio of pear-shaped to spindle-shaped neurons was 5.3:1 in the first, 15:1 in the second, 4.3:1 in the third, and 3.6:1 in the fourth spinal nucleus. Cone-shaped neurons were found only in one large larva, at positions 2 and 5 in the fourth spinal nucleus. Primary motor neurons were found in positions 2–4. Up to four bilaterally arborizing neurons were found at the anterior end of the first spinal nucleus (fig. 9).

D. quadramaculatus and *D. monticola*. A medial

and a lateral motor column were well developed in the smallest larvae studied (29.5 and 14.5 mm SVL, respectively), which were not recently hatched. Pear-shaped neurons were found in positions 1–3, with the majority in position 1. Spindle-shaped neurons were found in positions 3–5, with a concentration in position 5. The ratio of pear-shaped to spindle-shaped neurons was 1.25:1 in the first, 2.7:1 in the second and 2.4:1 in the third spinal nucleus. Cone-shaped neurons were always absent. Primary motor neurons were found only in the second spinal nucleus in positions 3–5 in larvae of both species. One to four bilaterally arborizing neurons were found at the rostral end of the first spinal nucleus.

D. ochrophaeus (fig. 10). A medial and a lateral motor column were present in the smallest larva studied (10 mm SVL), which had already resorbed its yolk. Pear-shaped neurons were present only in positions 1–3 in all nuclei, with the majority found in position 1 in the first spinal nucleus and in position 2 in the second spinal nucleus (fig. 10, 16c, d). In contrast, spindle-shaped neurons were found exclusively in positions 3–5, with the majority occurring in position 5 in the first spinal and in position 4 in the second spinal nucleus (fig. 16c, d). Thus, the neurons of the two motor columns overlap less in the first than in the second spinal nucleus. The ratio of pear-shaped to spindle-shaped neurons was 1.9:1 in the first, 4.7:1 in the second, and 1.9:1 in the third spinal nucleus. As in the other desmognathine species, no cone-shaped neurons were found in any nucleus of any individual. Primary motor neurons were found in positions 3–5 in the first and second nuclei of both larvae and adults. One to four bilaterally arborizing neurons were found at the rostral end of the first spinal nucleus (fig. 10).

D. aeneus (fig. 11). Medial and lateral motor columns were well developed in the smallest individual studied (13.2 mm SVL), which was a hatchling with its yolk sac still present (fig. 11). In fact, this hatchling possessed an essentially adult proportion (i.e., 50%) of spindle-shaped neurons (fig. 18). Pear-shaped neurons were found in positions 1–3 in the first and second spinal nuclei, with the majority found in position 1, whereas spindle-shaped neurons were found in positions 3–5, with a concentration in position 5 (fig. 15c). As in *D. ochrophaeus*, the neurons of the two motor columns overlap less in the first than in the second spinal nucleus. The ratio of pear-shaped to spindle-shaped neurons was 1:1 in the first and 10:1 in the second spinal nucleus. Only one cone-shaped neuron was found

in position 1. Primary motor neurons were found in the first spinal nucleus in both larvae and adults. Cone-shaped neurons were found in the first spinal nucleus in both larvae and adults.

P. jordani (fig. 12). Medial and lateral motor columns were well developed in the smallest larva studied (10 mm SVL), which was not recently hatched. Pear-shaped neurons were found in positions 1–3, with the majority in position 1. Spindle-shaped neurons were found in positions 3–5, with the majority in position 5. The ratio of pear-shaped to spindle-shaped neurons was 1.25:1 in the first, 2.7:1 in the second and 2.4:1 in the third spinal nucleus. Cone-shaped neurons were always absent. Primary motor neurons were found only in the second spinal nucleus in positions 3–5 in larvae of both species. One to four bilaterally arborizing neurons were found at the rostral end of the first spinal nucleus.

H. italicus (fig. 13). A medial and a lateral motor column were present in the smallest larva studied (10 mm SVL), which had already resorbed its yolk. Pear-shaped neurons were present only in positions 1–3 in all nuclei, with the majority found in position 1 in the first spinal nucleus and in position 2 in the second spinal nucleus (fig. 13, 16c, d). In contrast, spindle-shaped neurons were found exclusively in positions 3–5, with the majority occurring in position 5 in the first spinal and in position 4 in the second spinal nucleus (fig. 16c, d). Thus, the neurons of the two motor columns overlap less in the first than in the second spinal nucleus. The ratio of pear-shaped to spindle-shaped neurons was 1.9:1 in the first, 4.7:1 in the second, and 1.9:1 in the third spinal nucleus. As in the other desmognathine species, no cone-shaped neurons were found in any nucleus of any individual. Primary motor neurons were found in positions 3–5 in the first and second nuclei of both larvae and adults. One to four bilaterally arborizing neurons were found at the rostral end of the first spinal nucleus (fig. 10).

Discussion

A phylogenetic understanding of the development in salamanders of five types of primary motor neurons is important for the development of the primary motor column.

(1) Large primary motor neurons

in position 3 in the second spinal nucleus of an adult. Primary motor neurons were present in positions 3–4 in the first and second spinal nuclei of juveniles and adults. One to four bilaterally arborizing neurons were found at the rostral end of the first spinal nucleus in both hatchlings and adults (fig. 11).

P. jordani (fig. 12). Both motor columns were well developed in the smallest juvenile studied (17.5 mm SVL), which hatched at least 1 year before the experiment. Pear-shaped neurons were found in positions 1–3, with the majority approximately equally distributed between positions 1 and 2. Spindle-shaped neurons were found in positions 3–5, with a majority in position 5 (fig. 15d). The ratio of pear-shaped to spindle-shaped neurons was 1:1 in the first and 2:1 in the second spinal nucleus. There was one cone-shaped neuron in position 3 of the first spinal nucleus and two cone-shaped neurons were present in positions 3 and 4 of the second spinal nucleus. No primary motor neurons were found. Two to four bilaterally arborizing neurons were found at the rostral end of the first spinal nucleus (fig. 12).

H. italicus (fig. 13). In contrast to all other species described above, all developmental stages in this species lack a lateral motor column (fig. 13, 16e, f). Pear-shaped neurons were found at positions 1–4, with the majority found in position 2 in the first spinal and in position 1 in the second spinal nucleus (fig. 16e, f). Two pear-shaped neurons were found at position 5, at the border of the white matter. A few spindle-shaped neurons (15 of 813) were found in small individuals (≤ 21 mm SVL) in the first spinal nucleus at positions 3–5, at the border but not outside the gray matter. No cone-shaped neurons were present. One cell, appearing to be a primary motor neuron, was found in a juvenile at position 4 in the first spinal nucleus. All types of neurons are aligned much less regularly in this species than in adults of other species. No strongly bilaterally arborizing neurons were found (fig. 13).

Discussion

A phylogenetic perspective is necessary in order to understand the diversity of motor neuron development in salamanders. In the plesiomorphic condition, five types of motor neurons appear during the development of the anterior spinal cord.

(1) Large, multipolar neurons are most probably primary motor neurons, which are associated with

Mauthner axons and innervate only axial musculature in teleosts [Fetcho, 1986a]. At least some of these neurons survive metamorphosis and are found in adults of aquatic and semiaquatic amphibian species. These neurons are usually absent in terrestrial amphibians with direct development, such as the plethodontid tribes Plethodontini and Bolitoglossini among salamanders, and the large leptodactylid genus *Eleutherodactylus* among frogs [Hughes, 1959]. They may also be absent in species of *Bufo* and *Bombina*, because these species lack Mauthner neurons [Zottoli, 1978; Will, 1986], although at least one species of desmognathine salamander (i.e., *D. aeneus*) possesses primary motor neurons but not Mauthner neurons.

(2) Pear-shaped neurons, situated in the ventromedial part of the spinal gray matter, possess lateral, ventrolateral and contralateral dendritic arbors. These neurons are most probably the first to appear during the development of those species that lack primary motor neurons [Youngstrom, 1940; Nordlander, 1986]. The medialmost of these neurons are often undifferentiated and appear to be newborn cells [Fetcho, 1986a].

(3) Spindle-shaped neurons possess wide ipsilateral, but never contralateral dendritic arbors. These neurons are situated in the ventrolateral part of the spinal gray matter (often called the ventral horn) or within the adjacent white matter. These neurons increase in number and migrate laterally during embryonic and/or larval development. Neuron types 2 and 3 are secondary motor neurons that innervate both axial and appendicular musculature in urodele amphibians.

(4) No cone-shaped or (5) bilaterally arborizing neurons have been described in other works on spinal cord development in amphibians. The reason for this may be that both of these neuron types are restricted to the cervical spinal cord, whereas previous work has concentrated mostly on either limb or caudal regions. In salamanders, bilaterally arborizing neurons (*sensu stricto*) occur only in the anterior part of the first spinal nucleus, whereas cone-shaped neurons occur in all of the anterior spinal motor nuclei. We hypothesize that cone-shaped neurons are associated with innervation of neck musculature, based on: (1) morphological similarity between cone-shaped neurons and neurons of the accessory spinal nerve which innervate the neck muscle, *musculus cucullaris*, [Roth and Wake, 1985a]; (2) the fact that all of the four anterior spinal motor nuclei that contain cone-shaped neurons in-

nervate neck musculature, and (3) the fact that this neuronal type is apparently absent from the nuclei of more caudal spinal nerves. Studies using retrograde transport of HRP from neck muscles to the spinal cord will be necessary to verify this hypothesis.

Species of salamanders differ in patterns of spinal cord development. There is variation in the types of motor neurons that appear during development, and in the times of appearance of cell types relative to the time of hatching. This variation is associated with differences in life history and overall developmental patterns. The ten species of salamanders studied here fall into five groups that can be distinguished on the basis of their life history and developmental patterns. These groups differ accordingly in the development of their spinal motor neurons.

Group 1. Members of this group possess the plesiomorphic, or ancestral, pattern of development, from which the other groups have been derived during phylogenesis [Duellman and Trueb, 1986]. They have aquatic larvae, undergo metamorphosis and are aquatic or semiaquatic as juveniles and adults. *P. waltl*, *D. quadramaculatus*, *D. monticola*, *D. ochrophaeus*, *E. bislineata* and *P. ruber* belong to this group. All of these species are reported to possess Mauthner neurons throughout life [Fritzsche, 1981; Will, 1986], although we did not find somata of Mauthner neurons in the brainstem of our adult *D. ochrophaeus*. In addition, both larvae and adults possess a well-developed lateral line system [Hilton, 1947].

Most species of this group possess all five types of spinal motor neurons. The only exception is the genus *Desmognathus*, several species of which lack cone-shaped motor neurons. Primary motor neurons are present from the earliest to the latest larval stages, and are found in adults, at least in *D. ochrophaeus*.

In *Eurycea*, the only species in this group for which yolky hatchlings were examined, spindle-shaped neurons of the lateral motor column are not yet present at hatching (fig. 18). The lateral motor column begins to develop soon after hatching and reaches adult proportions before the onset of metamorphosis. In *Pleurodeles*, the lateral motor column develops faster than in *Eurycea*. In all species, spindle-shaped neurons of the lateral motor column increase in number, and become more lateral in position during posthatching ontogeny.

Group 2. Species of this group have larvae that are more fully developed at the time of hatching, and have a shorter larval period, than those of group 1

[Duellman and Trueb, 1986]. The representative of this group is *S. salamandra*. Juveniles and adults are terrestrial and possess neither Mauthner neurons nor a lateral line system, both of which are lost during metamorphosis [Fritzsche, 1981]. Primary motor neurons are present in larvae, but not in adults. The earliest larvae we studied, 1–2 days after hatching, already have some spindle-shaped neurons in the lateral motor column (fig. 18), and the proportion of spindle-shaped neurons is similar to that of adults at only 7 days after hatching (fig. 14). Thus, the development of the lateral motor column appears to be accelerated in this group compared to *Pleurodeles* and *Eurycea*.

Group 3. Species of this group, represented here by *D. aeneus*, have direct development with no aquatic larval stage. Metamorphosis takes place inside the terrestrial egg, and both juveniles and adults are fully terrestrial. Mauthner neurons are absent in *D. aeneus* [Nishikawa, unpubl. observations], but an anterior lateral line nerve and central projections are present, although neuromasts have yet to be found [Nishikawa et al., unpubl. observations]. Curiously, primary motor neurons are present and persist to adulthood despite the absence of these related input systems. In *D. aeneus*, the development of the lateral motor column is even more accelerated than in *Salamandra*. A well-developed lateral motor column, with adult proportions of spindle-shaped neurons, is present in yolky hatchlings (fig. 18).

Group 4. This group includes direct-developing species of the plethodontid tribe Plethodontini, represented here by *P. jordani*. In contrast to group 3, juveniles and adults of these species possess no remnants of aquatic life. They do possess some larval characters transiently in embryonic development, but these are lost before hatching [Dent, 1942]. Both Mauthner neurons and the lateral line system are absent. In these species, no primary motor neurons are found at posthatching stages. Small juveniles, more than 1 year old, possess a well-developed lateral motor column. Unfortunately, embryos and hatchlings of groups 4 and 5 are difficult to obtain and were not included in this study.

Group 5. This group includes members of the plethodontid tribe Bolitoglossini, represented here by *H. italicus*. These species are characterized by a different type of direct development, termed ontogenetic repatterning [Roth and Wake, 1985b]. Ontogenetic repatterning is a pattern of development, like pedomorphosis or neoteny, in which some embryonic char-

acteristics of neoteny and are a novel character.

In *Hydromantes* species still present a lateral motor column. Bolitoglossini is present not only a lateral motor column are aligned in contrast, both lateral motor neurons orientation observed glossine spindle-shaped neurons we found shaped neurons a bolitoglossini cone-shaped so absent.

The plesiomorphic species of *D. aeneus* group. In these group most plesiomorphic followed by derived organisms and manders were also lack s arborizing

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acteristics are retained in adults. However, unlike neoteny and paedomorphosis, repatterned ontogenies are a mosaic of paedomorphic, peramorphic and novel characteristics.

In *Hydromantes* and all of the other bolitoglossine species studies so far, a lateral motor column is never present at any developmental stage. Only a medial motor column, consisting of pear-shaped and polygonal neurons located exclusively inside the gray matter, is present. In adult nonbolitoglossine salamanders, not only are cell bodies aligned in medial and lateral motor columns, but the dendrites of these neurons are aligned in a mediolateral direction as well. In contrast, both the cell bodies and dendritic trees of spinal motor neurons in adult bolitoglossines vary in their orientation. The lack of alignment is similar to that observed in early posthatching stages of nonbolitoglossine species, except that bolitoglossines lack spindle-shaped neurons. In some juveniles, and in 1 adult, we found 1-2 neurons that resemble the spindle-shaped neurons of other salamanders. However, these neurons are always found within the gray matter. In bolitoglossine salamanders, primary motor neurons, cone-shaped and bilaterally arborizing neurons are also absent.

The plesiomorphic ontogeny is modified in most species of direct-developing amphibians (except *D. aeneus*), in that no primary motor neurons develop. In these species, the first motor neurons to develop most probably are small, pear-shaped neurons, followed by large spindle-shaped neurons. The most derived ontogeny is found in the bolitoglossine salamanders which, in addition to primary motor neurons, also lack spindle-shaped, cone-shaped and bilaterally arborizing neurons.

Unlike the highly paedomorphic, ontogenetically repatterned bolitoglossine salamanders [Wake, 1966; Roth and Wake, 1985b], the neotenic, perennibranchiate salamanders, such as *Ambystoma mexicanum* (axolotl) and *Necturus maculosus* (mudpuppy), show the plesiomorphic set of motor neurons. This is because these forms have a normal developmental pathway which is stopped at a late larval stage, by which time all of the spinal motor neuron types are already present. In contrast, plethodontine and especially bolitoglossine salamanders show a more or less complete lack of larval neuron types, and substantial repatterning of the developmental process. This indicates the importance of the distinction between neoteny (i.e., reproduction in the larval stage) and

paedomorphosis (i.e., any retardation of development, including but not restricted to neoteny), with or without ontogenetic repatterning. Paedomorphosis may involve much more profound retardation of development than neoteny, in that paedomorphic structures may never develop beyond early embryonic stages.

The evolutionary pattern that emerges from these studies is that derived species of salamanders generally possess fewer different types of motor neurons than plesiomorphic species. Salamanders generally show a much lower degree of morphological differentiation and cell migration than anuran amphibians and other vertebrates, with the exception of lepidosirenid lungfishes [Northcutt, 1987]. Bolitoglossine salamanders exhibit an even lower degree of morphological differentiation and migration than other salamanders [Roth, 1987; Linke and Roth, 1989, 1990; Roth et al., 1990]. This is the case in the retina and optic nerve [Linke and Roth, 1989, 1990], the mesencephalic tectum [Roth, 1987; Roth et al., 1990] and thalamic, pretectal and tegmental nuclei [Wicht and Himstedt, 1988; Naujoks-Manteuffel and Manteuffel, 1988]. In addition, medial motor neurons fail to migrate into a lateral position outside the gray matter during development. The lack of a lateral motor column in the spinal cord of bolitoglossines is apparently part of the overall reduction of cell migration in the nervous system of these species. The mechanism of suppression of cell migration in salamanders has not yet been elucidated.

Two general patterns characterize the posthatching ontogeny of cervical spinal motor neurons in all species studied. First, there is a rostrocaudal gradient in the development of cervical spinal motor neurons in all species for which data are available (fig. 16a-f). The gradient is expressed both in the proportion of differentiated neurons, and in the degree of cell migration. There is a higher proportion of spindle-shaped neurons in more anterior nuclei than in more posterior nuclei, and in general, neurons occupy a more medial position in more posterior nuclei, suggesting that less cell migration has occurred. For this reason, the motor columns overlap more in more posterior nuclei.

Second, of the five motor neuron types present in the anterior spinal cord of salamanders, only spindle-shaped neurons increase in number, in relative proportion, and in the distance of somata from the central canal between hatching and adulthood. All of the

other neuron types are present in hatchlings, juveniles and larvae in the same positions and approximately the same proportions as in adults. Furthermore, in all species studied, pear-shaped neurons are found almost exclusively in medial, periventricular positions 1-3, whereas spindle-shaped neurons are found only laterally (with a single exception, fig. 15b) at the border or just outside of the white matter at positions 3-5. Spindle-shaped neurons are located more laterally at later developmental stages. These observations suggest that the spindle-shaped neurons of the lateral motor column are derived from medially born pear-shaped neurons, which acquire their characteristic morphology during their outward migration as they reach the border of the white matter.

A remaining question is at what point during ontogeny the fate of spindle-shaped neurons is determined. There are two possibilities: (1) that pear-shaped neurons, despite apparent morphological uniformity, consist of two distinct cell lineages from birth, i.e., those that will remain pear-shaped and will not migrate laterally, and those that will both migrate and differentiate into spindle-shaped neurons, or (2) that some pear-shaped neurons are determined epigenetically at a later point in development to migrate and differentiate into spindle-shaped neurons. To date, no experimental evidence allows us to distinguish between these two possibilities.

It is interesting to note the existence of another major difference between pear-shaped and spindle-shaped neurons that is characteristic of all of the species studied: most pear-shaped neurons possess some contralaterally projecting dendrites, whereas the dendrites of spindle-shaped neurons ramify almost exclusively in the ipsilateral white matter.

Amphibian motor neurons have been classified based on: (1) their time of appearance (e.g., primary vs. secondary motor neurons), (2) their morphology (e.g., multipolar vs. bipolar vs. unipolar), (3) their targets (e.g., axial vs. limb muscles), and (4) their position in the spinal cord (e.g., lateral vs. medial motor columns). In amniotes and frogs, there appears to be a general correspondence among the attributes used to classify motor neurons. For example, the neurons of the lateral motor column are lateral, spindle-shaped neurons that innervate muscles of the limb [Fetcho, 1987]. Amniotes that lack limbs, such as snakes, also lack a lateral motor column [Fetcho, 1986b], and in most limbed vertebrates, including frogs, the lateral motor column is larger in the brach-

ial and sacral regions than elsewhere [Silver, 1942]. In salamanders, however, the association among attributes of motor neurons is different from that of amniotes. For example, although they possess limbs, neither brachial nor sacral enlargements of the spinal cord are present in salamanders [Roth and Wake, 1985a].

The lateral and medial motor columns of salamanders do not correspond to limb versus axial muscle targets, as they do in amniotes. Stephens and Holder [1985] studied the motor columns innervating the limb muscles of the axolotl (*A. mexicanum*). They found two pools of motor neurons: large spindle-shaped neurons situated laterally within or just outside the gray matter, and small, medial neurons in the periventricular gray matter, the former being much more numerous than the latter. Retrograde labeling of axial or limb muscles or their respective nerves always labeled both types of neurons.

Other studies of salamanders have also found that axial muscles are innervated by both types of neurons [Fetcho, 1986a; Roth and Wake, 1985a; Wake et al., 1988; Nishikawa, unpubl. observations]. Thus, the amniote view that the medial motor column innervates axial musculature and the lateral motor column innervates limb musculature does not apply to urodele amphibians.

An alternative hypothesis for the functions of the motor columns in anamniotes is that medial and lateral motor neurons innervate different populations of muscle fibers. In teleosts, small, ventrolateral neurons innervate both red and white muscle fibers, whereas large, dorsomedial neurons innervate only white muscle fibers [Fetcho, 1986a, 1987]. Unfortunately, no such data exist for amphibians, although both frogs and salamanders possess red and white muscle fibers [Sasaki, 1974; Totland, 1976a, b].

In amphibians, the first muscle fibers to appear during ontogeny are red fibers [Kordylewski, 1986]. However, it is not known whether later appearing white fibers are formed de novo or are transformed slow fibers. Furthermore, it is not known whether the appearance of a specific type of motor neuron induces the formation of white fibers. In *Xenopus*, the axial muscles consist predominantly of red fibers early in development, and the proportion of white fibers increases with age [Kordylewski, 1986]. Conversely, the primary motor neurons believed to innervate only white fibers appear earliest in ontogeny [Fetcho, 1987], while secondary motor neurons, believed to in-

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nervate both types of muscle fibers, increase in number during later ontogeny. In *Xenopus*, the earliest primary motor neurons appear at Nieuwkoop and Faber stage 24/25 [van Mier et al., 1985], the earliest secondary motor neurons are present by stage 39 [Nordlander, 1986], and the earliest differentiated axial muscle fibers of either type are not present until stage 48 [Kordylewski, 1986]. Thus, in *Xenopus*, there appears to be no close association between the appearance of different motor neuron types and muscle fiber types during ontogeny.

Both amniotes and salamanders possess discrete, nonoverlapping medial and lateral motor columns, based on the position of motor neurons within the spinal cord. Furthermore, the morphology of the pear-shaped neurons that constitute the medial motor column and the spindle-shaped neurons that constitute the lateral motor column are similar in the two taxa. However, the targets of these neurons differ between taxa. This implies a lack of homology, and therefore a lack of developmental similarity, between the superficially similar motor columns.

Having demonstrated that salamanders and amniotes must differ in their patterns of motor neuron development, the remaining question is: in which taxa have the evolutionary transitions occurred during phylogeny? One possibility is that tetrapods invented target-specific motor columns at the same time that they invented limbs, and that salamanders, being pedomorphic, have reverted to a more primitive pattern of motor neuron development, in which some axial as well as some (but not all) limb motor neurons migrate outside the spinal gray matter during ontogeny.

An alternative possibility is that target-specific motor columns evolved in amniotes, long after the evolutionary appearance of limbs. If this hypothesis is true, the frogs should have motor columns that are more similar to those of salamanders than those of amniotes. Some anecdotal evidence from frogs supports this hypothesis. Nishikawa and Wassersug [1988] observed that HRP application to tail muscles of *Xenopus* resulted in staining of both medial and lateral motor neurons, although this observation was not published in the original report. Fetcho and Reich [1989] also suggest that the segregation of epaxial and hypaxial motor pools is an invention of amniotes, and thus evolved long after the appearance of limbs.

While these observations are suggestive, a systematic study is necessary before either of these alternatives can be rejected. Future studies of the morphoge-

nesis and migration of limb and axial motor neurons in amphibians versus amniotes should prove interesting from both developmental and evolutionary perspectives.

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