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Morphology of the Caudal Spinal Cord in *Rana* (Ranidae) and *Xenopus* (Pipidae) Tadpoles

KIISA NISHIKAWA AND RICHARD WASSERSUG
Museum of Vertebrate Zoology, University of California, Berkeley, California 94720 (K.N.), and Department of Anatomy, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada (R.W.)

ABSTRACT

Using a variety of neuroanatomical and histological techniques, we compare the spinal cord and peripheral nerve distribution in the tails of larvae from *Xenopus laevis* and three species of *Rana*.

The relatively large, postsacral spinal cord of *Xenopus* contains abundant motoneurons and their axons. Spinal nerves exit from the spinal cord in a regular array, one nerve per myotome, from the cervical region to near the end of the tail. Somata of motoneurons innervating caudal myotomes are found along the entire length of the tail. In contrast, the caudal cord of *Rana* is reduced to a filum terminale consisting of little more than an ependymal tube; spinal nerves to all caudal myotomes leave the cord in the sacral region and reach their motor targets via a cauda equina and caudal plexus. Motoneuron cell bodies innervating caudal myotomes are found only in the sacral region.

The *Rana* larval pattern is similar to that of adult frogs and mammals, whereas the *Xenopus* larval pattern is more like that of salamanders and reptiles. These gross neuroanatomical differences are not due to differences in the size or developmental stage of the tadpoles, but instead are associated with differences in the swimming behavior of the larvae.

The presence of motoneurons in the caudal spinal cord of *Xenopus* may provide local intermyotomal control within the tail; the elongated topography of the cord appears to permit finer, rostral-to-caudal regulation of neuromuscular activity. The *Rana* spinal cord, on the other hand—with motoneurons clustered anteriorly—may produce concurrent firing of adjacent ipsilateral myotomes, but at the expense of fine intermyotomal regulation. The fact that nerves in the tail of *Xenopus* enter and exit from the spinal cord locally, as opposed to far anteriorly as in *Rana*, means that for tadpoles of the same size, reflex arc lengths are many times shorter in *Xenopus*.

Key words: motoneurons, myotomes, locomotion, tail, amphibians

Anuran larvae and embryos have a relatively simple locomotor system compared to that of most fishes and tetrapods (Roberts, in press; Wassersug, in press; Fetcho, '87), and consequently often serve as models in studies of the neural control and development of vertebrate locomotion (for recent examples see Roberts et al., '83, Stehouwer and Farel, '85; van Mier et al., '85; Westerfield and Eisen '85; Nordlander, '86; Roberts, in press).

Although more than 300 genera of frogs are currently recognized (Frost, '85), two genera alone—*Rana* and *Xenopus*—account for approximately one-third of all that we know about anuran biology, and three quarters of what we know about their neurobiology.[1] Because of their long history in biological research, *Rana* and *Xenopus* are, by default, the "typical" anurans. In this report we describe

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Address reprint requests to Kiisa Nishikawa, who is now at the School of Biological Sciences, University of Kentucky, Lexington, KY 40506.

[1]The first statement is based on an unpublished analysis of citations from the last decade of the *Zoological Record*; the second is based on references to genus names found in the text of *Frog Neurobiology* (Llinás and Precht, '76). Of 927 references counted, 684 (74%) were for either *Rana* or *Xenopus*.

major neuroanatomical differences between *Rana* and *Xenopus* larvae that are associated with differences in their locomotor behavior.

Behavioral differences between *Rana* and *Xenopus* larvae are well documented (Wassersug, in press). During constant-velocity rectilinear locomotion, *Rana* tadpoles generate propulsive waves in their tails by firing myotomes in the body and anterior portion of the tail only (Hoff, '87); waves of bending observed in the posterior tail do not result from muscle contractions but travel passively in whiplike fashion (Blight, '77; Hoff, '87). *Xenopus* larvae, in contrast, swim by rapidly vibrating the filamentous tip of their tails. Except when they turn or swim fast (i.e., at speeds >6 body lengths s^{-1}; Hoff and Wassersug, '86), movement in the *Xenopus* tail is confined to the tail tip and no waves of bending are visible in the tail anteriorly.

Rana and *Xenopus* larvae also differ fundamentally in the way that they regulate swimming velocity. In *Rana*, velocity changes in a linear fashion with changes in tail beat frequency (Wassersug and Hoff, '85), but the length of the propulsive wave in the tail does not change with swimming speed. In *Xenopus*, on the other hand, a constant tail beat frequency is maintained over a broad range of swimming velocities, and *Xenopus* increases speed by increasing the length of the propulsive wave in the tail, i.e., the amount of the tail that is bent (Hoff and Wassersug, '86). As *Xenopus* larvae swim faster, they incorporate more and more of their tail in the power stroke. Only for velocities above approximately 6 bodylengths s^{-1} (where the length of the propulsive wave approaches the tail length) must *Xenopus* increase tail beat frequency, like *Rana*, in order to swim faster.

The present study began as an effort to understand what morphological factors, if any, relate to these behavioral and kinematic differences. We began by exploring the distribution of motoneurons and the pattern of spinal nerve distribution in the two genera. Of course, *Rana* and *Xenopus* larvae differ in many nonneural morphological features, such as body and tail fin shape (see figures in Wager, '65) as well as musculoskeletal histology (e.g., Khajeh Dalooi, '77; Nishikawa et al., '85; Hoff and Wassersug, '86; Hoff, pers. comm.). However, these differences cannot explain the different ways in which these larvae regulate swimming velocity. In this report we show that *Xenopus* and *Rana* tadpoles differ radically in the organization of the caudal spinal cord and suggest that this difference underlies the fundamental difference in the way larvae of the two genera coordinate axial motor activity.

MATERIALS AND METHODS

We examined three anatomical features of the spinal cord in larval *Xenopus* and *Rana*: 1) gross diameter, including relative amount of white matter and number of cell somata, from midtrunk to the end of the tail, 2) position of emerging spinal nerves in relation to caudal myotomes, and 3) the location and distribution of motoneurons innervating myotomes in different parts of the tail.

Species with tadpoles of similar size were selected for examination: *Rana pipiens, Rana sylvatica* (Ranidae), and *Xenopus laevis* (Pipidae). *Rana catesbeiana* larvae, which are generally larger than *X. laevis*, were also examined.

All *Xenopus* larvae were raised from eggs laid by adults bred in the laboratory. *Rana* larvae were collected from ponds in the vicinity of Halifax, Nova Scotia, or purchased from a commercial supplier in California. Tadpoles were maintained at room temperature in aerated aquaria for up to several weeks before study. They were fed standard laboratory diets of baker's yeast cells in aqueous suspension (for *Xenopus*) or boiled lettuce (for *Rana*). Under these conditions, most tadpoles behaved and grew normally. Only healthy individuals, growing at normal rates without scoliosis or damaged tail fins, were examined. All tadpoles were staged according to Gosner ('60) and their total lengths measured to the nearest 0.5 mm. Unless otherwise noted, all comparisons between species are for individuals matched for size and developmental stage.

At least five specimens of each species, ranging from Gosner stages 25–43 (equivalent to Nieuwkoop and Faber, '56, stages 46–63 for *Xenopus*; and Taylor and Kollros, '46, stages I–XXII for *Rana*) were embedded in paraffin and cut in frontal and transverse section at 10 μm. Transverse sections were stained with haematoxylin, phloxine, and saffron. Frontal sections were stained alternately with cresyl violet and Palmgren's silver stain (Humason, '79). The diameter of the spinal cord was measured from sections through the central canal at its widest point, at several intervals along the length of the tadpole. In these sections, the width of the white matter was measured on both sides of the central canal. The number of cell somata between the central canal and the white matter on both sides of the spinal cord were recorded. All types of cells, including ependymal cells, interneurons, and motor neurons, were counted.

To observe the distribution of axons to the periphery, tadpoles were fixed in neutral buffered formalin, skinned, eviscerated, and stained with Sudan black following the method of Nishikawa ('87). This technique stains all peripheral myelinated nerves without destroying collagenous myosepta. All caudal spinal nerves were traced from the spinal cord to their neuromuscular junctions in caudal myotomes. Camera lucida drawings of the spinal nerves were made from these Sudan black preparations. In order to document inter- and intraspecific variation, four specimens of *R. pipiens* and five of *R. sylvatica* were examined, with the latter representing a developmental series ranging from posthatching (Gosner stage 26) to near-metamorphosis (Gosner stage 42).

The locations and distributions of motor neurons innervating cervical, sacral, and postsacral caudal myotomes were investigated by using retrograde transport of horseradish peroxidase (HRP). For each species, at least seven specimens were studied. They were anesthetized with 0.25% tricaine methane sulfonate (MS222, Sigma Chemical Co., St. Louis, MO). The skin above a single myotome on each side was cut and the underlying muscle tissue between neighboring myosepta was macerated with forceps. In preliminary studies we found that HRP was not taken up by muscles that were not macerated. A slurry of crystalline HRP (Sigma type VI; Sigma Chemical Co.) dissolved in 2% dimethylsulfoxide was placed in epaxial myotomes of the 2nd–20th postotic segments. HRP applications were made in different myotomes along the rostrocaudal axis on the left and right sides of each tadpole. During a postsurgical survival period of two to five days, the HRP was transported retrogradely along the axons to motoneuron somata in the spinal cord. After the survival period, specimens were overanesthetized with 1.0% MS222 and perfused with saline followed by fixative (2.8% glutaraldehyde, 1.0% paraformaldehyde, and 2.5% sucrose in 0.12 M phosphate buffer, pH 7.4). Following perfusion, the spinal cords were removed, postfixed for 15–30 minutes and placed in 0.1 M

cacodylate buffer at pH 5.45 for 2 hours. Whole spinal cords were incubated in 0.2% diaminobenzidine (Sigma Chemical Co.) and 0.1% hydrogen peroxide in 0.1 M cacodylate buffer (pH 5.45) for 30 minutes, then dehydrated in graded alcohols and cleared in methyl salicylate.

RESULTS

Gross differences in the dimensions and components of the caudal spinal cord of *Rana* and *Xenopus* are illustrated in Figures 1–4. Both *Rana* and *Xenopus* larvae have distinct cervical and lumbar enlargements containing motor pools for muscles of the developing fore and hind limbs. The two genera differ slightly in the relative position along the rostrocaudal axis of the lumbar enlargements, in part because of the slightly longer presacral vertebral column in *Xenopus*.

More significantly, the cords differ in their relative thickness along the rostrocaudal axis. In the presacral region, the spinal cord of *Rana* is larger in diameter than that of *Xenopus* (Fig. 1). In the postsacral region, the *Rana* cord narrows abruptly and becomes one-half to one-fifth as wide as the *Xenopus* cord. In *Rana*, the conus medullaris lies between 40% and 70% of the distance along the rostrocaudal axis from the otic capsules to the tail tip; in this region the cord decreases tenfold in diameter. As one moves caudally, the *Xenopus* cord, in contrast, decreases in diameter neither as much nor as abruptly as it does in *Rana*. The difference between the widest and narrowest parts of the *Xenopus* larval cord, from the front of the trunk to midtail, is only two- to threefold.

The more abrupt tapering of the spinal cord in *Rana* is due to differences in the distribution of both cell bodies (gray matter) and motor axons in the spinal cords of the two genera. There is a much more abrupt and more extreme diminution in both the number of cell bodies and the amount of white matter in the *Rana* cord lateral to the central canal compared to the *Xenopus* cord (Figs. 2, 3). The *Rana* cord narrows to a single ependymal layer in the first third of the tail (Figs. 2, 4), whereas the *Xenopus* cord remains larger and motoneurons are clearly present past 80% of its length. Very little white matter is present in the *Rana* cord past 60% of tail length (Figs. 3, 4). Palmgren and cresyl violet stained specimens show no myelinated axons in the spinal cord of *Rana* past 70% of tail length, whereas in the *Xenopus* cord myelinated axons are present all the way to the tail tip (Fig. 4).

These results raise the question of where the motoneurons lie for caudal myotomes of *Rana*. Our HRP experiments indicate that motoneurons for myotomes as far posterior as the 19th postotic segment have their cell bodies in the trunk region of the cord (Table 1, Fig. 5). We found in all cases that motoneuron somata labeled from caudal myotomes in *Rana* were located anterior to the conus medullaris at the level of the 8–11th spinal roots. In contrast, in *Xenopus*, motoneurons innervating the 15th and more

Abbreviations

a	axons	sg	spinal ganglion
ec	ependymal cell	sm	secondary motoneuron
n	notochord	ss	segmental spinal nerve
nc	neural canal	wm	white matter
pm	primary motoneuron	7th-V	seventh vertebra
rb	Rohon Beard cell		

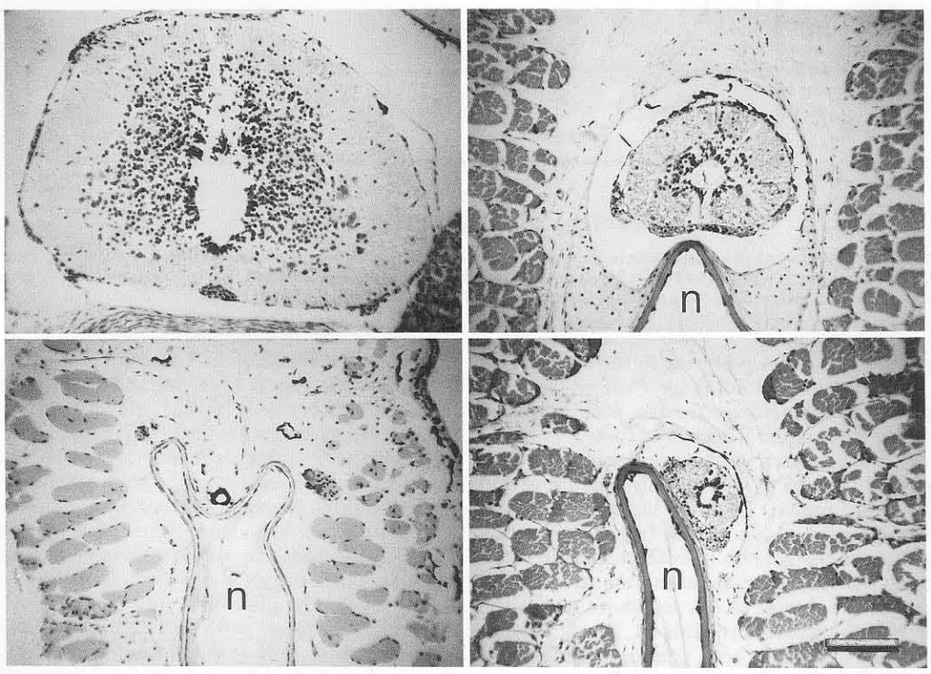

Fig. 1. Photomicrographs of cross sections of spinal cords stained with haematoxylin, saffron, and phloxine, from *R. pipiens* (left) and *X. laevis* (right) larvae of identical length and similar premetamorphic stage. Top sections are from the trunk region at 40% of total length from snout to tail tip. Lower sections are from the midtail region at 78% of total length. All photomicrographs are reproduced at similar magnification (scale lines equal 0.1 mm) in order to reveal absolute differences in size. Note particularly the small size of the caudal spinal cord of *Rana* (lower left) (see also Fig. 4).

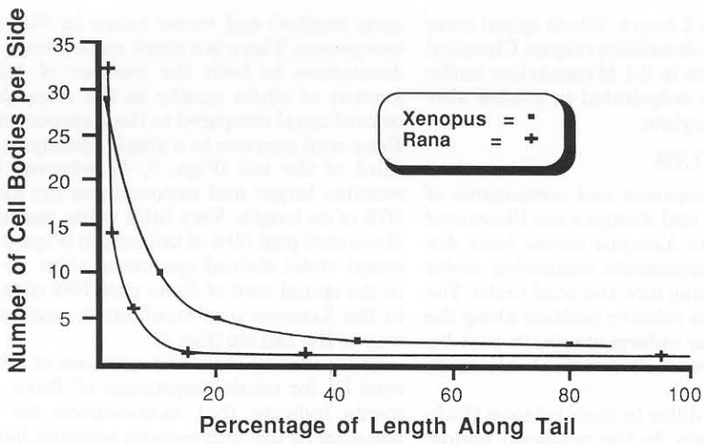

Fig. 2. The number of cell bodies lateral to the central canal in frontal sections of *R. sylvatica* and *X. laevis* spinal cords plotted against percentage of length along tail from the lumbar enlargement (0% on the X-axis = the widest point of the lumbar expansion) to the tail tip (100% on X-axis). Data collected from cresyl violet stained 10 μ frontal sections; specimens matched for size and stage (Gosner stage 39). Note that at 15% of total tail length the cellular component of the *Rana* cord is reduced to a single ependymal cell. *Xenopus*, in contrast, retains at least one motoneuron on each side of the caudal cord past 80% of its length, and the cord narrows to less than two somata per side at approximately 40% of tail length.

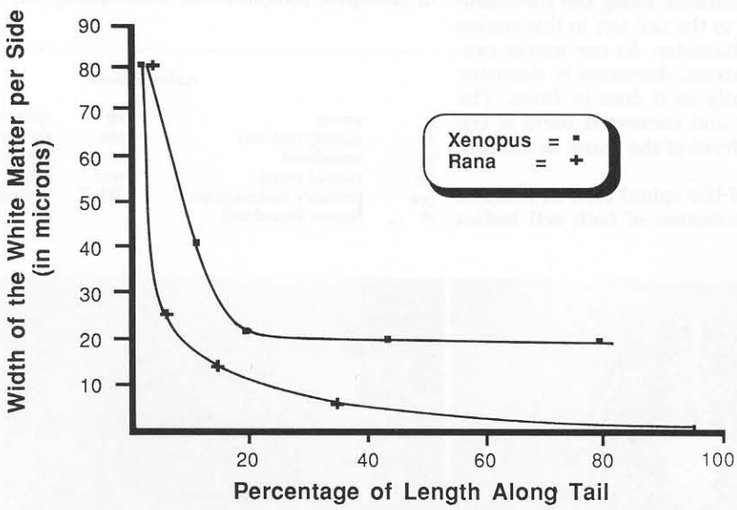

Fig. 3. The absolute width of white matter in *Rana catesbeiana* and *Xenopus laevis* spinal cords at the level of the central canal. Data collected as in Figure 2. Note that although both species have the same amount of white matter at the lumbar enlargement (80 μ per side), the amount of white matter diminishes more rapidly and more extensively in *Rana* than in *Xenopus*.

posterior myotomes are found posterior to the conus. This corroborates the results of Nordlander ('86) on younger larvae in which the motoneurons for the 40th myotome were located at the level of the 37th spinal root.

The basic vertebrate pattern of one spinal nerve for each metameric segment (Fetcho, '87) is seen in both *Rana* and *Xenopus* in the trunk region. There is, however, a consistent caudal displacement of the nerves in relation to the myotomes posterior to the sacrum in both genera; in other words, myotomes in which nerves terminate are usually several body segments caudal to the segments where the nerves enter or exit the spinal cord (Table 1, Fig. 5; see also Westerfield and Eisen, '85). In both genera, this displacement is accentuated as one progresses caudally.

In both genera myotomes are multiply innervated (Fig. 5). Nordlander ('86) reports that the motor column serving an individual myotome in *Xenopus* spreads over four spinal segments. We found that the rostrocaudal extent of the motor column innervating a single myotome increases from one to two segments in the trunk region to three or more segments near midtail (see length of the black bars in Fig. 5). In *Rana* the rostrocaudal extent of the motor columns along the same length of the spinal cord varies less and was never found to exceed more than two spinal segments.

Gross differences in peripheral nerve patterns are shown in Sudan black preparations (Figs. 6–9). Individual segmental nerves can be identified only for the first 11 postsacral metameres in *Rana*, with little intra- or interspecific variation (range = 1, N = 9) and no ontogenetic variation in the number of identifiable segmental nerves. Caudal to the 11th myotome we did not observe a one-to-one correspondence between myotomes and spinal nerves. Instead, in the

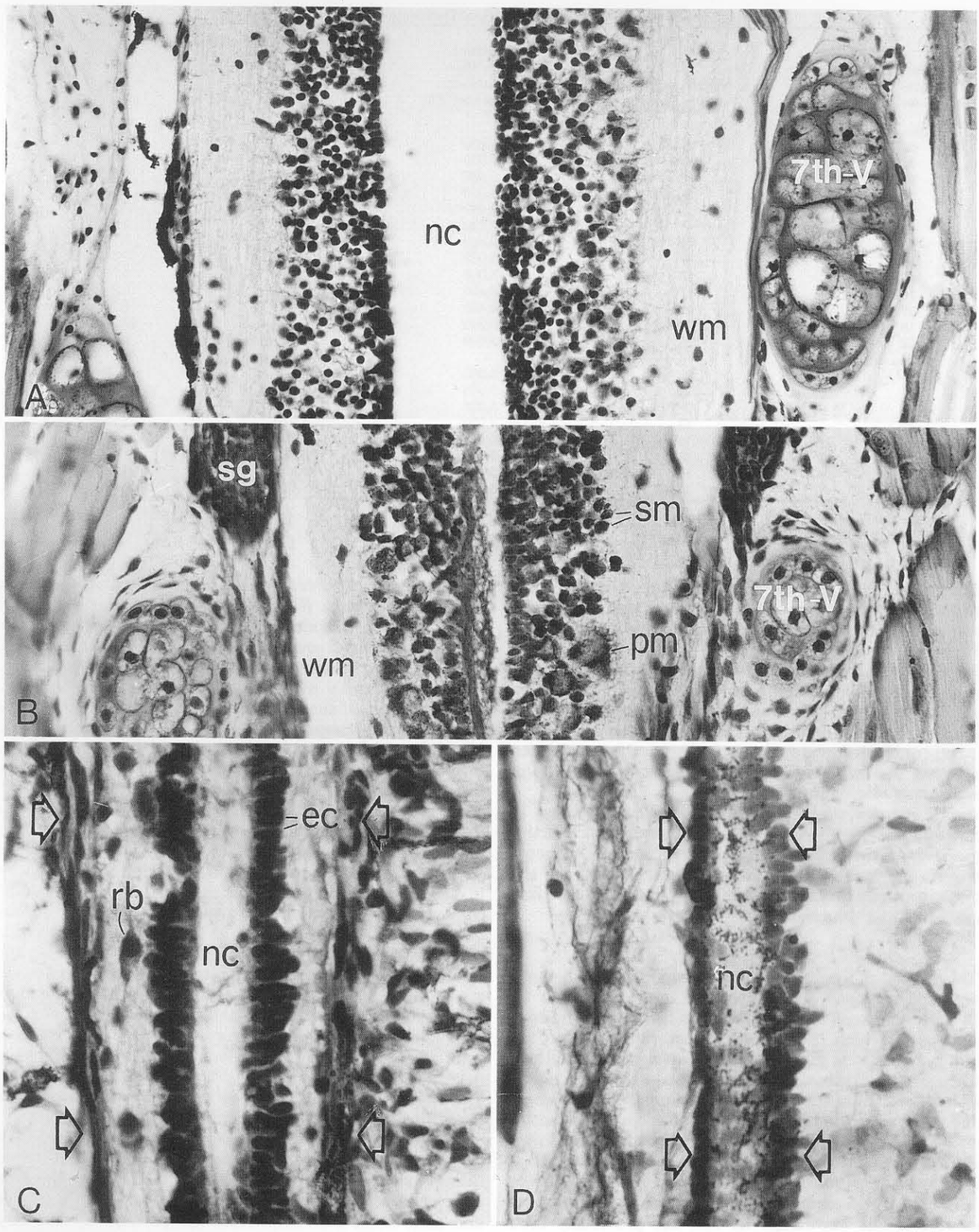

Fig. 4. Photomicrographs of frontal sections of *X. laevis* (A, C) and *R. sylvatica* (B, D) larval (Gosner stages 37–39) spinal cords, stained with cresyl violet. Total tail lengths equal 20 mm for both specimens. The top sections are from the trunk region at the level of the 7th vertebra. The lower sections are from the midtail region at 70–75% of total tail length. The top photomicrographs are reproduced at 150×, lower ones at 260×. Arrows in lower figures indicate pia mater. Note absolute differences in the size of the caudal spinal cord between species. Axons are absent from the caudal spinal cord of *Rana*, (D), which is little more than an ependymal tube (see also Figs. 2,3).

TABLE 1. Site of Application of HRP and Its Final Distribution Within the Spinal Cord*

Postotic myotome labeled	Ventral spinal roots labeled	Distance from obex to lumbar enlargement (#)†	Distance from obex to: anterior end of motor pool (#)	Distance from obex to: posterior end of motor pool (#)
Xenopus laevis				
2	2–3	2,800	140	660
9	8–9	2,800	1,500	2,260
9	8	2,700	1,500	2,080
9	9–10	3,600	2,640	3,680
10	10–11	2,400	2,000	3,540
11	11	2,700	2,760	3,600
12	12	2,400	2,780	3,740
14	14	3,600	4,620	5,440
15	15	2,000	3,000	4,020
16	15	2,400	3,720	5,200
17	17	2,400	4,060	5,280
18	19–20	3,240	9,160	10,920
20	20	2,000	5,480	6,540
Rana catesbeiana				
2	1	5,000	−540	+60
11	10–11	5,000	3,060	4,640
11	9–10	5,300	3,700	4,820
12	12	4,900	4,240	5,960
14	14	5,400	5,700	6,000
16	16	5,800	6,880	7,500
16	16	5,400	6,100	6,840
19	17–18	5,000	7,100	8,000

*See also Figure 5.
†Given as an index of body size.

midportion of the *Rana* tail, the nerves form a cauda equina and the spinal cord is reduced to a filum terminale. Each nerve passes many myotomes forming a plexuslike pattern (Fig. 9).

Xenopus retains the one-to-one topographic correspondence of spinal nerves to myotomes along the length of the tail. This one-to-one corresponding segmentation can be identified well past midtail, to the 29th postsacral myotome in Sudan black preparations. Only in the caudalmost quarter of the tail does it become difficult to match spinal nerves with specific myotomes.

Axons of individual motoneurons, particularly those innervating posterior myotomes, leave the spinal cord farther anteriorly in *Rana* than in *Xenopus*. As a result, although absolute reflex arc lengths are similar in the anterior tail, in the posterior tail arc lengths may be up to 20 times longer in *Rana* than in *Xenopus*.

DISCUSSION

The gross differences in spinal cord morphology between *Rana* and *Xenopus* are comparable, respectively, to the differences between the spinal cords of mammals (and adult anurans) in which there is a distinct cauda equina and filum terminale, and that of salamanders and reptiles in which an intact spinal cord with segmental spinal nerves extends well into the tail (Nieuwenhuys, '64). Whereas some features of the larval *Rana* and *Xenopus* spinal cords that we have noted have been observed before (see Brown, '46, re the caudal plexus in *Rana*; Khajeh Dalooi, '77, re the segmental spinal nerves in *Xenopus*; Filoni and Bosco, '81, re the large diameter of the caudal cord in *Xenopus*), to the best of our knowledge no one has previously documented that the postsacral spinal cord in *Rana* larvae is a filum terminale formed from only an ependymal tube, whereas the *Xenopus* larval cord includes motoneuron somata for neighboring caudal myotomes and extends virtually the full length of the tail.

Intermyotomal control of tail bending during swimming is not as precise in *Rana* as in *Xenopus* (Wassersug and Hoff, '85; Hoff, '87), since *Rana* modulates velocity by controlling tail beat frequency anteriorly, rather than by differential firing of one mytome in relation to its neighbors. *Xenopus* swims by movement in the caudal rather than rostral portion of the tail and alters velocity (via changes in the length of propulsive wave in the tail) by differential recruitment of myotomes, so that precise control of one myotome in relation to its neighbors is essential. Two neuroanatomical features may relate directly to these differences in locomotor control. For one, *Rana*, motoneurons for myotomes are packed in the sacral region of the conus medullaris, anterior to the filum terminale. If these closely packed motoneurons are electrically coupled or otherwise in close synaptic association (as are some other topographically clustered motoneurons in the frog spinal cord—Sonnhof et al., '77; Brenowitz et al., '83), such organization would produce nearly simultaneous firing of myotomes and would not generate rostrocaudal lags in neural activity that are associated with fine intermyotomal control. The elongate topography of the motoneurons in the *Xenopus* tail would, in contrast, produce the rostrocaudal lags that characterize differential control of one myotome relative to its neighbors. Ultrastructural studies are necessary to test these hypotheses concerning the dendritic terminals of motoneurons in *Rana* and *Xenopus*.

A second neuroanatomical feature is the persistence of a one-to-one correspondence between myotomes and spinal nerves far caudally in *Xenopus*, suggesting that, theoretically at least, intermyotomal control is both more precise and more local in *Xenopus* (except perhaps at the very terminus of the tail; see below). The fact that motoneurons in much of the *Xenopus* tail reside relatively close to the myotomes they innervate means that the absolute length of reflex arcs is much shorter in *Xenopus* than in *Rana*. Hoff ('87) has calculated that for tadpoles of 8 cm total

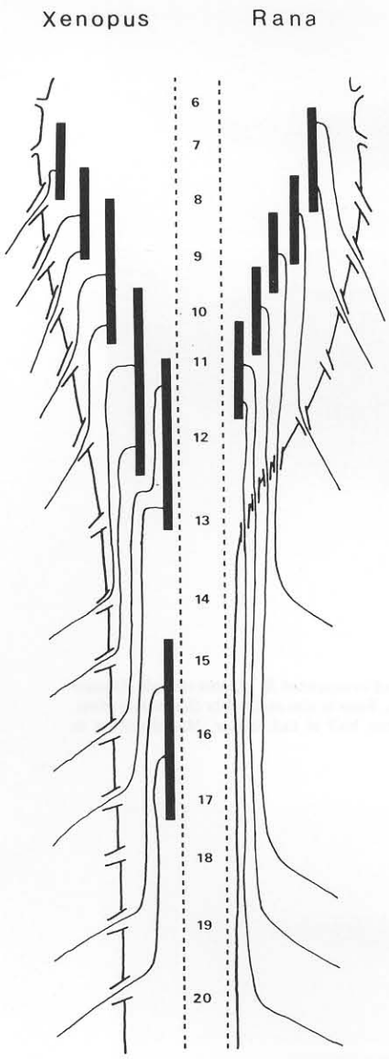

Fig. 5. Schematic drawing summarizing horseradish peroxidase experiments on *X. laevis* and *R. catesbeiana* (Gosner stages 32–39). One-half of the spinal cord from the level of the 6th to the 20th postotic myotome is illustrated for *Xenopus* (left) and *Rana* (right). Spinal nerve levels are given at the midline. Axons from the spinal cord to specific myotomes are drawn as light lines laterally exiting the cord. Vertical black bars indicate the rostrocaudal extent of the region of motoneuron somata filled with HRP transported retrogradely from specific myotomes. Quantitive data are given in Table 1. Not all myotomes were treated with HRP, as indicated by the gap opposite the 14th myotome in the *Xenopus* series. Note that the caudal spinal cord is wider in *Xenopus* than in *Rana* and contains motoneurons. Other differences are discussed in the text.

length, the arc lengths at midtail are approximately 3.1 times longer in *Rana* than in *Xenopus*; this translates into conduction time differences of as much as 11 msec if we use myelinated nerve conduction velocities for anurans (Stämpfli and Hille, '76) and assume similar axonal diameters and myelination between species. Hoff ('87) and Stehouwer and Farel ('80, '81) support the idea that peripheral feedback strongly influences the regulation of swimming in anuran larvae. Electrophysiological studies are necessary to determine whether or not there are significant differences in conduction velocity in *Rana* and *Xenopus*.

Isolated *Xenopus* tails can oscillate with "actual swimming movements" (Shaffer, '63; also Tata, '66) for at least a week in tissue culture, whereas *Rana* tails do not exhibit this in vitro behavior (pers. obs. R.W.). This difference suggests that the central pattern generator for swimming movements in *Xenopus* is, indeed, more local in *Xenopus* than in *Rana*. Shaffer ('63) reports complete reflex arcs within the caudal tail of *Xenopus* larvae.

Differences in development underlie the anatomical differences that we have noted. *Xenopus* larvae continually add new myotomes to the end of the tail as they grow (Nordlander, '86). Consistent with this is the observation by Nordlander ('86) that the spinal cord continues to grow from its terminus in larval *Xenopus* and to progressively differentiate as more tail and spinal cord are added on. In contrast, lengthening of the tail in *Rana* is reflected more in an increase in the size of individual myotomes than in an increase in myotome number (Hoff, pers. comm.) and little if any neurodifferentiation takes place in the filum terminale of *Rana*. Unlike *Xenopus*, the tail of *Rana* cannot be viewed as a continuous developmental series in which the terminus shows the earliest developmental stages.

The incremental, terminal addition to the *Xenopus* tail presents a particular problem relating to the neuromuscular regulation of the oscillating tail tip. The first neurons to form at the tail tip are, by definition, primary neurons (Nordlander, '86). Primary motoneurons (as discussed and reviewed by Fetcho, '87) innervate only white muscle in fishes and, presumably, amphibians. Yet, at the tail tip of *Xenopus* the oscillating muscle is exclusively small red fibers (Kordylewski, '86). It may be that terminal red muscle fibers are innervated by neither primary nor secondary motoneurons, but are electrically coupled (see Hayes, '75). This is consistent with the fact that the tail tip has a fixed, intrinsic oscillating frequency (c. 10 Hz at 21°C; Hoff and Wassersug, '86). In this case, the oscillating muscle of the tail tip must lose its myogenic properties as it is incorporated in the tail during growth (as has been proposed for trunk muscle in embryonic amphibians by Stehouwer and Farel, '80). The terminal muscle evidently serves as the progenitor for both the superfical red and the deep, white muscle of the tail (Sasaki, '74; Watanabe et al., '78).

As a final point, the neuroanatomical differences between *Rana* and *Xenopus* spinal cords may reflect evolutionary history as well as differences in the control of locomotion. *Rana* and *Xenopus* are not closely related among anurans, for they are placed in separate suborders by all current workers (e.g., Frost, '85; Duellman and Trueb, '86). The caudal spinal cord morphology in *Xenopus* is more generalized and the *Rana* pattern more derived when compared to other vertebrates, such as salamanders and primitive fishes. Larvae of certain clearly archaic frogs, such as *Ascaphus truei* and discoglossids, however, swim like *Rana*. Other clearly derived anurans, including many treefrog larvae within the family Hylidae, swim like *Xenopus*. The question of the extent to which differences in spinal cord morphology between *Rana* and *Xenopus* larvae reflect evolutionary history will be resolved only with comparative study of spinal cords in a wider taxonomic array of larvae.

ACKNOWLEDGMENTS

This investigation follows from functional morphology research begun in collaboration with Karin v.S. Hoff; we

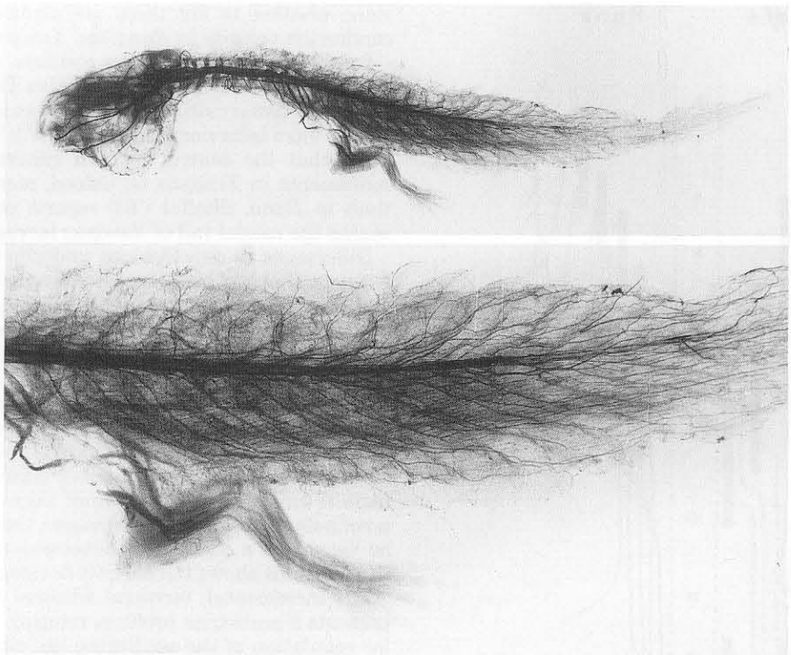

Fig. 6. Cleared and stained Sudan black preparation of a skinned and eviscerated *R. pipiens* tadpole (Gosner stage 40) in glycerine showing the myelinated peripheral nervous sytem. Bone is also stained in this preparation. Whole specimen, above; closeup of posterior half of trunk and anterior half of tail, below. Magnification is approximately 2× for the whole specimen and 5× for the closeup.

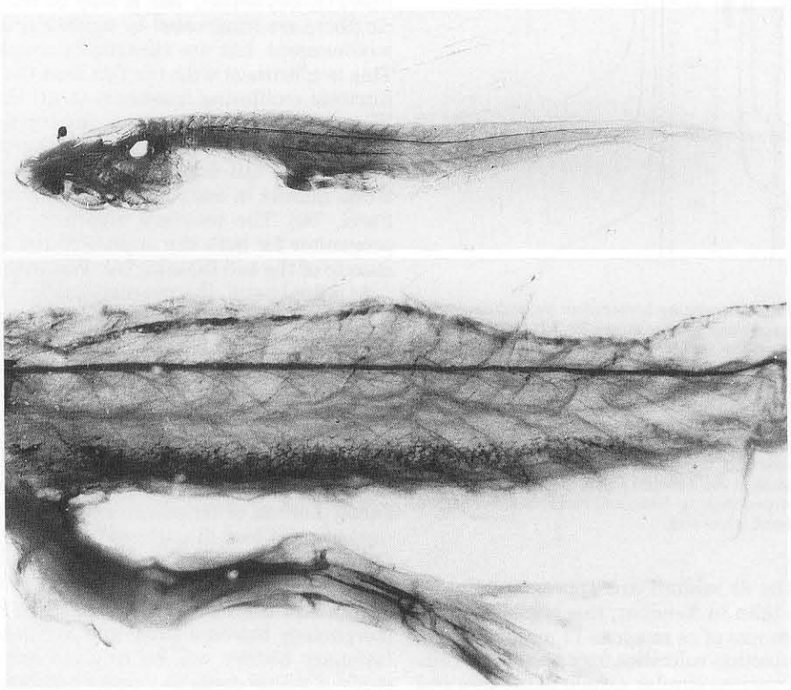

Fig. 7. Cleared and stained Sudan black preparation of a *X. laevis* tadpole (Gosner stage 40); preparation, photography, and magnification similar to that for the *R. pipiens* larva shown in Figure 6.

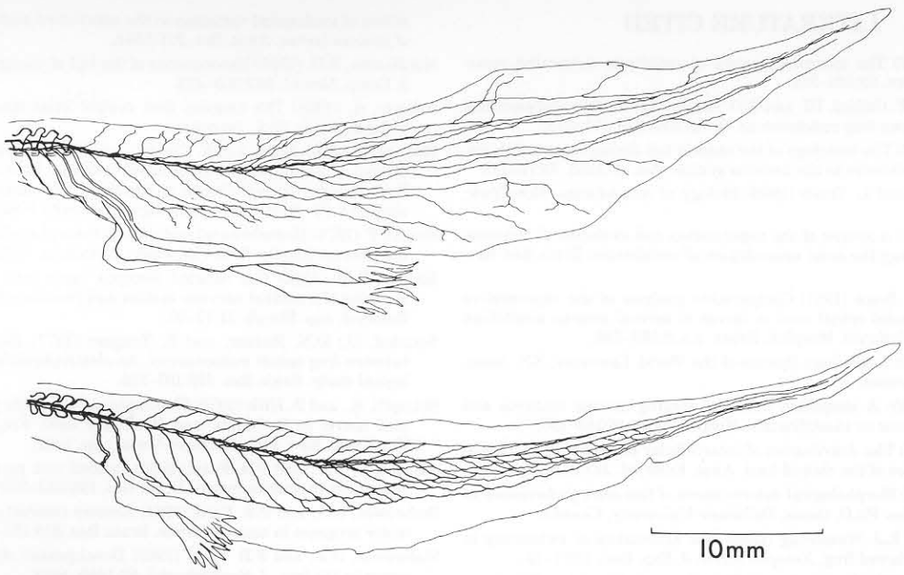

Fig. 8. Camera lucida drawings of the nerves in the tails of *R. pipiens*, (above) and *X. laevis* (below) from Sudan black preparations, such as those shown in Figures 6,7. Only major nerves (i.e, primary, secondary, tertiary branches) are illustrated; terminal branches of nerves in the tail fin of *Xenopus* are not illustrated.

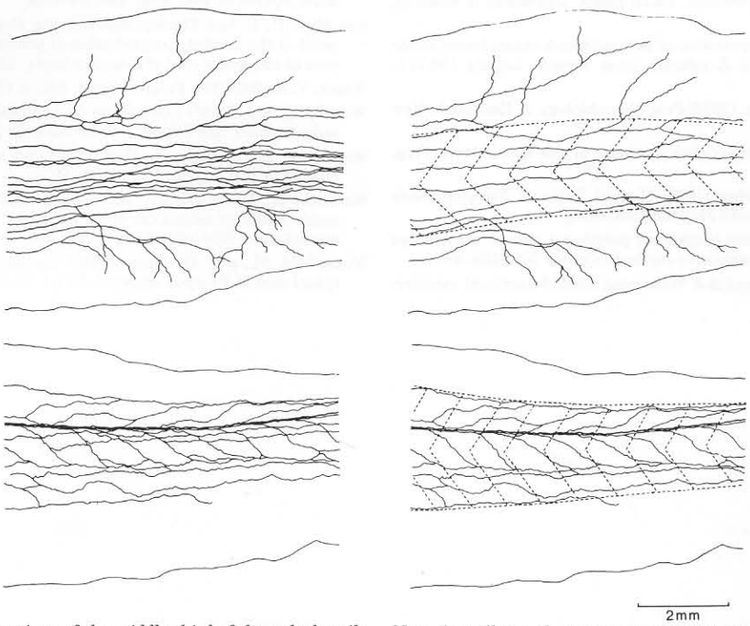

Fig. 9. Camera lucida drawings of the middle third of the tadpole tails illustrated in Figures 6–8; *Rana* (above), *Xenopus* (below). All stained nerves are illustrated in the lefthand figures. Figures on right are for the same regions from the same preparations, but here myotome boundaries are drawn in (as dotted lines) and only primary nerve branches are illustrated. Note that all spinal nerves originate anterior to the illustrated region in the *Rana* tail and course past many myotomes, whereas in *Xenopus* spinal nerves exit the cord locally. In *Xenopus* there is a one-to-one correspondence between the number of myotomes and the number of spinal nerves in this region.

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