

Tongue Evolution in Lungless Salamanders, Family Plethodontidae.

III. Patterns of Peripheral Innervation

DAVID B. WAKE, GERHARD ROTH, AND MARVALEE H. WAKE
Museum of Vertebrate Zoology and Department of Zoology, University of California, Berkeley, California 94720, (D.B.W., M.H.W.) and Department of Biology, University of Bremen, 2800 Bremen 33, Federal Republic of Germany (G.R.)

ABSTRACT Innervation of the tongue and associated musculature in plethodontid salamanders was studied using Palmgren stained sectioned materials, fresh dissection, and whole mounts of experimental specimens treated with horseradish peroxidase (HRP). Species studied were chosen to represent modes of tongue projection recognized by Lombard and Wake ('77). Special attention was given to species of the genera *Plethodon*, *Batrachoseps*, *Pseudoeurycea*, and *Hydromantes*, but representatives of other genera were investigated. As expected we found that cranial nerves IX and X and spinal nerve 1 supplied the muscles involved in tongue movement. The peripheral courses of the nerves were traced, and both functionally related and phylogenetically determined routes were found. As relative projection length increases, the nerves supplying the tongue tip also increase in length. When the tongue is at rest the long nerves are stored in coils. The coil of ramus lingualis lies between the ceratobranchials, but that of ramus hypoglossus is more variable, although constant within a species. Ramus hypoglossus bifurcates into separate branches to tongue and anterior musculature of the floor of the mouth. In generalized, presumably primitive, modes the bifurcation and coiling are far anterior. In most of the tongue projection modes bifurcation is relatively posterior, but in one, bifurcation is anterior, but coiling is relatively posterior in position. The most unusual condition is in *Hydromantes*, in which bifurcation is relatively posterior and a coiled ramus hypoglossus joins a coiled ramus lingualis to form a unique, coiled common ramus to the tongue tip. *Hydromantes* has the greatest projection distance of any salamander.

When this series of papers was initiated, a sequence of three publications was envisioned. The first dealt with the methodology to be employed, and treated the problem from a theoretical viewpoint (Lombard and Wake, '76). The second was a comparative analysis, using the theoretical paper and a model contained therein as a point of departure (Lombard and Wake, '77). The project subsequently has expanded, and publications not in the series (Roth, '76; Thexton et al., '77; Wake and Lombard, '71; Wake, '82) have contributed to the general topic. Accordingly we extend the series by adding new information on the pattern of peripheral innervation of the tongue and associated structures. A gen-

eral phylogenetic analysis and additional contributions in this series are in progress.

Choice of species for this study was made in order to permit two levels of analysis. First, we required a morphocline with respect to degree of tongue freedom and projection. *Plethodon* and *Aneides* are generalized with tongues attached at the front of the mouth, and they have relatively slight projection capability. *Ensatina* and *Hemidactylium* have looser anterior attachments and somewhat more projection capability. *Batrachoseps* has even more anterior freedom, but retains a loose anterior attachment. It has more projection capability than does *Ensatina*. *Pseudoeurycea*, *Pseudotriton*, *Eurycea*,

and *Hydromantes* all lack an anterior attachment and have complete anterior freedom. *Pseudotriton* appears to have the least projection capability of this group, with *Eurycea* and *Pseudoeurycea* having intermediate levels of projection. *Hydromantes* has the most highly projectile tongue of any salamander.

Second, we required a careful selection of species so that we could control for phylogenetic effect. All of our species are members of the subfamily Plethodontinae. *Plethodon* and *Aneides* (functional tongue mode II of Lombard and Wake, '77) and *Ensatina* (mode III) comprise the tribe Plethodontini of Wake ('66). *Eurycea* and *Pseudotriton* (mode IV) and *Hemidactylium* (mode V) are members of the tribe Hemidactyliini. *Bolitoglossa* and *Pseudoeurycea* (mode VI), *Hydromantes* (mode VII), and *Batrachoseps* (mode VIII) represent the three supergenera that comprise the tribe Bolitoglossini. We chose not to study representatives of mode I (attached-tongue members of the subfamily Desmognathinae) at this time, because they are similar to mode II.

MATERIALS AND METHODS

A variety of techniques was employed to determine patterns of innervation of tongue elements. One to 20 (*Batrachoseps attenuatus*) representatives of each species were dissected to determine gross pattern. Courses of nerves and their points of innervation of muscles, muscle arrangements, shapes of cartilages, and positions of ligaments were reconstructed graphically from transverse serial sections of whole heads. Heads were fixed in Duboscq's alcohol-formalin-picric acid-glacial acetic acid, decalcified in HCl-alcohol, embedded and sectioned, and stained by the Palmgren method according to standard procedures. Courses of specific nerves were determined by anaesthetizing animals in MS222, then cutting the nerves and applying crystalline HRP to the stumps. After a survival time of 2 to 6 days, the animals were anaesthetized, perfused with 0.8% NaCl solution until all blood was washed out, then perfused with 2% glutaraldehyde, 2% paraformaldehyde, and 2.5% sucrose in 0.12 M phosphate buffer (pH 7.4) until the animal was stiff (about 15 minutes). Horseradish peroxidase is transported in the nerve fibers during the survival period. The reaction product of HRP and diaminobenzidine is brown-black, rendering the anterograde and

retrograde courses of nerves visible. The brain was excised and the lower jaw, tongue, and hyoid apparatus were removed. Incubation was similar to that described by Fritzsche ('81). Fixative was washed out with 0.12 M cacodylate buffer (pH 5.45) for at least 1 hour. Brains and tongues were then immersed for 1 hour in cacodylate buffer with 0.2% diaminobenzidine and 0.01% H₂O₂ (the latter added in drops in 3% solution). Whole mounts were prepared by dehydrating in graded alcohols to 100% EtOH, and then they were immersed in cedarwood oil and photographed. Other preparations were made by embedding in paraffin and sectioning according to standard methods.

The primary subjects of this study are members of the genera *Plethodon*, *Batrachoseps*, *Pseudoeurycea*, and *Hydromantes*, for which we have experimental data and serial sections. Less complete data are available for other genera (in most instances, only from dissection).

DEVELOPMENTAL AND PHYLOGENETIC BACKGROUND

We will restrict our attention to the nerve supply to the musculature that is involved directly in movement of the tongue during feeding. We will not deal with jaw opening and closing muscles, or ventral throat constrictors. The tongue muscles can be organized into two groups from a developmental and neuroanatomical viewpoint: the hypobranchial muscles supplied by somatic motor neurons, and the branchiomeric muscles supplied by visceral motor neurons. While these muscles have been studied by many workers, and the urodele pattern is generally well known, previous studies have been mainly descriptive or so broadly comparative that the comparisons have little value for phylogenetic analysis. Further, no comparative analyses of plethodontids are available. The few studies that have included any neuroanatomical information (Bowers, 1900; Szamoylenko, '04; Magimel-Pelonnier, '24) are incomplete and inaccurate. Finally, no experimental studies using modern tracer techniques have been used in studies of urodele tongue muscles.

Good summaries of the developmental and adult morphology of the tongue musculature of urodeles may be found in Francis ('34) and Fox ('54), both of whom built on the foundation of work by Drüner ('01, '04). None of

these authors considered plethodontid material directly, except to comment on the results of Bowers (1900). The most directly relevant publications are those of Piatt ('35, '38); however, Piatt's developmental work was restricted to ambystomatids. The only plethodontid that has been studied in any detail is *Eurycea bislineata* (Bowers, 1900, for cranial nerves; Smith '20, for muscles before and during metamorphosis; Wake and Lawson '73, for anterior spinal nerves), but these studies are incomplete. Szamoylenko ('04) studied *Hydromantes italicus*, but many of her observations are erroneous.

From these background sources the following picture emerges. The hypobranchial muscles are derived from the first two or three postotic somites, and are supplied by the first two spinal nerves. As in other urodeles, the geniohyoideus medialis and genioglossus muscles are supplied by the ramus hypoglossus. The geniohyoideus lateralis, apparently unique to ambystomatoid salamanders (Piatt, '40), is supplied by this ramus also (*contra* Szamoylenko, '04; see below), as expected from Smith's ('20) discovery that this muscle and the geniohyoideus medialis have a common anlage. The hyoglossus, lying entirely in the tongue pad, also is served by the ramus hypoglossus. Because the hyoglossus develops late, during metamorphosis, its origins usually have been surmised. Similarly, the other tongue pad muscles (circumglossus, basiradialis, interradianalis) are thought to be supplied by the same ramus. The basiradialis and interradianalis are associated with radii, cartilages of dubious homology in plethodontids. If the radii are derived from the hyoid arch, as is generally assumed, it is possible that these muscles might be of branchiomic origin and served by the ramus lingualis of the glossopharyngeal nerve, known to supply the sensory innervation of the tongue pad and known to give off motor innervation on its way to the tongue. However, no larval or embryonic precursors of branchiomic muscles have been observed in this region. Further, if the radii were derivatives of the hyoid arch, one would expect facial rather than glossopharyngeal innervation of their muscles (although there is a ramus communicans between facial and glossopharyngeal nerves in urodeles). If the radii are part of the subhyoid system, as argued by Jarvik ('63), hypobranchial origin of the muscles is expected.

Thus, the developmental origin of the tongue pad muscles remains unknown, but it has been assumed that they arise from the anterior part of the hypoglossal musculature (Piatt, '38).

The rectus cervicis-rectus abdominis series of muscles in plethodontids is unique (Lombard and Wake, '77). In generalized plethodontids, the rectus cervicis superficialis is always present as a distinct element, and the rectus cervicis profundus and rectus abdominis profundus are joined into a single muscle that is separated into two parts at the level of the sternum by a myocomma. In more specialized forms this myocomma is absent, and the muscle is uninterrupted from its origin on the ischium to its insertion in the tongue pad. Generalized species have a rectus cervicis superficialis lateralis that extends from the sternum to the anteroventral margin of the first ceratobranchial. This muscle is joined behind the urohyal by the small omohyoideus. In these same species a heboosteopsiloideus is present, extending from a myocomma in the rectus cervicis-abdominis profundus at the level of the sternum to the urohyal. In the most specialized species only two muscles of the above complex remain: the rectus cervicis superficialis and the uninterrupted rectus cervicis-abdominis profundus.

The primary innervation of the hypobranchial musculature is the first spinal nerve. The next spinal nerve, the first that has both dorsal and ventral roots and a ganglion, is known to anastomose with the first spinal in *Eurycea* (Wake and Lawson, '73), as in other urodeles (Francis, '34; Fox, '54). In addition to the main ramus, there are other twigs to the posterior muscles.

The only branchiomic muscle of interest to us is the complex subarcualis rectus I. This muscle forms at metamorphosis from the larval muscle of the same name (Piatt, '38). In larvae it is served only by the glossopharyngeus, but in adults it has a vagal innervation as well (Francis, '34).

The glossopharyngeus is a large nerve that gives rise to a trunk issuing from the common ganglion of the glossopharyngeus and vagus. This trunk gives off a major branch to the portion of the subarcualis rectus I that is wrapped around the epibranchial, producing a muscular bulb (Lombard and Wake, '76). The nerve proceeds through the anterior parallel-fibered parts of that muscle, giving off

twigs until, as the ramus lingualis, it enters the tongue proper and finally reaches the pad.

The vagus is simplified in plethodontids as compared with other salamanders, for several reasons. First, of course, the plethodontids are all lungless and lack a larynx. Second, branchial reduction is evident in the family, with larval or developmental epibranchials reduced from four (in desmognathines) to three and finally one (various plethodontines; Wake, '66). Fox ('54) found evidence of seven branchial vagal nerves in *Andrias*. In fully metamorphosed salamanders the usual condition is three (Francis, '34): two branchial nerves and a wide-ranging ramus intestinalis-accessorius. These are present in plethodontids, and the first branchial vagal nerve serves the posterior part of the subarcualis rectus I. There may be a ramus communicans between the ramus intestinalis-accessorius and the first spinal nerve (Fox, '54).

No single phylogenetic hypothesis concerning urodeles can be defended strongly (Edwards, '76; Hecht and Edwards, '77). Much of the difficulty arises from the fact that several urodele families contain only perenni-branchiate or incompletely metamorphosed forms. Accordingly, it is difficult to make consistent comparisons across all families. For example, the tongue musculature and skeleton are built partially out of gill arch materials that only become available at metamorphosis. Fortunately, there are some generally well-defended hypotheses concerning the families that do contain fully metamorphosed species: Hynobiidae, Dicamptodontidae, Salamandridae, Ambystomatidae, and Plethodontidae. The Hynobiidae is the most primitive (plesiomorphic) of the five, having such ancestral features as a discrete angular bone in the lower jaw, external fertilization, large numbers of chromosomes (including microchromosomes), and all spinal nerves exiting between vertebrae (for other characters see Hecht and Edwards, '77). Hynobiids also have a primitive tongue (Regal, '66). The four remaining families are more derived and have more specialized tongues. Plethodontids and ambystomatids resemble one another in features of the hyobranchial apparatus, notably in having a distinct geniohyoideus lateralis muscle (a synapomorphy) absent in the other families. Further, these two families lack any sign of the subhyoideus muscles, which are stout, probably

plesiomorphic structures that are functionally important, at least in the Salamandridae and Hynobiidae.

In view of the above considerations, we have chosen salamandrids, and especially the species *Salamandra salamandra*, as an out-group for our analysis. When appropriate, we will also draw comparisons with members of the families Ambystomatidae and Dicamptodontidae. *Salamandra salamandra* has been available to us for study, and in addition there is a long tradition of morphological work on this species, and its tongue structure is particularly well known (summarized by Francis, '34; see also Özeti and Wake, '69).

Because we are especially interested in the evolution of projectile tongues within the family Plethodontidae, it is important for us to establish a logical framework for our comparisons within that family. There are four major taxonomic groups of plethodontids: subfamily Desmognathinae and the tribes Hemidactyliini, Plethodontini, and Bolitoglossini of the subfamily Plethodontinae (Wake, '66). There are eight major modes of tongue projection within the Plethodontidae (Lombard and Wake, '77); six of these are associated with tongue projection patterns, but the other two are characteristic of species with relatively nonprojectile tongues. All desmognathines have nonprojectile tongues like two of the three genera of the Plethodontini (*Plethodon* and *Aneides*). Two of the projectile modes are found among hemidactyliine genera, one in the Plethodontini, and three are found in the Bolitoglossini (corresponding to the three supergenera *Hydromantes*, *Batrachoseps*, and *Bolitoglossa*).

Desmognathines resemble *Plethodon* and *Aneides* in many features of tongue structure, and these groups in turn are the plethodontids that resemble nonplethodontids most closely in tongue morphology. We will not consider the desmognathines further in this paper, but have chosen to use *Plethodon* as an out-group, within the Plethodontidae, for the six modes of tongue projection.

GENERAL PATTERN OF INNERVATION

Before embarking on our detailed analysis, we present some basic information concerning musculature and associated innervation in *Salamandra* and *Plethodon*.

The first two spinal nerves in both *Salamandra* and *Plethodon* arise in moderately close proximity. The first, comprising only a ventral root and lacking a ganglion in meta-

morphosed individuals, exits through a foramen in the atlas vertebra, immediately behind the atlantal cotyle and in front of the neural pedicel rudiment. The second has both dorsal and ventral roots, and a relatively small ganglion. The ventral root exits through the anterior part of the wall of the second vertebra (first trunk vertebra), or between the first two vertebrae (Edwards, '76). The two ventral roots send rami ventrally, then posterolaterally, in parallel courses, and as they approach the rectus musculature there is a communicating ramus between them. This is part of what has been called the cervical plexus, a series of interconnections between the vagus and spinal 1, spinal 2, and spinal 2 and spinal 3 (see Francis, '34). The last does not concern us in this paper. The first, sometimes called the spino-occipitalis, is a controversial connection that we have not seen in any plethodontid. Francis ('34) said that it is present but difficult to find in *Salamandra*, where it lies deep in musculature.

The literature concerning the posterior cranial and anterior spinal nerves in urodeles is rather confusing. For example, Francis counted a transient nerve appearing briefly during the development of *Salamandra* as spinal nerve 1, and thus counts the first spinal nerve in adults as spinal nerve 2. Fox ('54) thus cited Francis ('34) as stating that the ramus hypoglossus (= ramus hypobranchialis) of *Salamandra* is formed from spinal nerves 2 and 3. There is no question concerning the homology of the first few spinal nerves in any urodeles, however, and because the first spinal nerve invariably pierces the atlas behind the atlantal cotyles in all urodele species, it should always be called spinal nerve 1. Drüner's ('04) statement that the ramus hypoglossus is formed from the first three spinal nerves apparently takes into account the small communicating ramus between spinal nerves 2 and 3.

Spinal nerve 1 contributes the majority of fibers to the ramus hypoglossus, which may be considered to start at the point at which the communicating ramus from spinal nerve 2 joins spinal nerve 1. A short distance from its origin, the ramus hypoglossus gives off a branch that, in turn, bifurcates to supply the rectus cervicis profundus and rectus cervicis superficialis. The main ramus moves sharply anteriorly as soon as it reaches its ventralmost point and runs along the subarcualis rectus 1 into the floor of the mouth. A short distance

after passing the urohyal the ramus gives off the first of several short branches that serve the geniohyoideus in *Salamandra* and the geniohyoideus medialis in *Plethodon* (these muscles are exact homologues). The ramus extends anteriorly along the lateral margin of the geniohyoideus, deep to the transverse throat muscles (interhyoideus and intermandibularis series), and just ventral to the anterior parts of the subarcualis rectus I and ceratohyal.

In *Salamandra* the ramus moves medially as the anterior end of the ceratohyal is reached, then moves dorsally, bifurcates, and enters the tongue pad far anteriorly. The anteriormost branch serves the genioglossus, and the posteriormost branch serves the hypoglossus (at least) and most probably the other tongue pad musculature as well.

In *Plethodon* the ramus hypoglossus gives rise to a major lateral branch before the anterior end of the ceratohyal is reached. This branch extends laterally as the main nerve supply of the geniohyoideus lateralis. There may also be some smaller twigs of the ramus that arise more posteriorly to serve this muscle. This is a controversial muscle that has engendered much discussion in the literature. It is clear that it is not the homologue of the subhyoideus of *Salamandra*; it is supplied by the ramus hypoglossus rather than the facialis, and it has a myotomal embryological origin (from a geniohyoideus anlage in *Eurycea* according to Smith, '20; from a genioglossus anlage in *Ambystoma* according to Piatt, '38). Francis ('34) suggested that this muscle might be essentially identical with some aberrant lateral slips of the geniohyoideus found by him and by Drüner ('04) in *Salamandra*, but these slips are oriented ventrally relative to the ceratohyal rather than dorsally as in the plethodontids. Our finding is apparently the first report that these muscles are supplied by the ramus hypoglossus, although such a discovery was anticipated by Piatt ('38), reasoning from embryological evidence. Bowers (1900) was unaware of this muscle, and Szamoylenko ('04), who first applied the name geniohyoideus lateralis to the muscle in plethodontids, incorrectly reported that both this muscle and the subhyoideus in *Salamandra* were supplied by the glossopharyngeus, whereas neither muscle is.

The second spinal nerve has several branches that supply neck musculature, but we are concerned only with the branch that

continues ventromedially, beyond the commissure with the first spinal, toward the rectus abdominis musculature. This branch bifurcates, with one part supplying the rectus cervicis superficialis and the other the rectus cervicis profundus. According to Francis ('34), in *Salamandra* a small branch leaves the main nerve just before the ramus communicans and supplies the omohyoideus, but we have not seen this. In *Plethodon*, where the rectus cervicis and rectus abdominis profundus are almost totally unified, the nervous supply of this compound muscle is from both the first and second spinal nerves, but in *Salamandra* the supply of the rectus cervicis musculature is from the second spinal only. We have been unable to find the nerve supply to the omohyoideus-rectus cervicis superficialis lateralis complex in *Plethodon*, because these muscles are very small. We believe the innervation is from the second spinal, as in *Salamandra*, for we have searched the first spinal and its branches without finding any branch to these muscles.

The glossopharyngeus has similar paths in *Salamandra* and *Plethodon*. The nerve arises separately by a single trunk from the anterior portion of the hind brain and enters the anterior part of the large ganglion shared by the glossopharyngeal and vagal nerves. The nerve exits from the anterior edge of the ganglion and courses ventromedially as a large, rather convoluted trunk. This trunk enters the large subarcualis rectus I muscle, which in *Plethodon* is partly wrapped around the epibranchial at this point. A large branch is given off to the musculature near the point of entrance, and the main trunk continues anteromedially, at first in the muscle and then along its outer boundary. The trunk gives off several small branches to the anterior part of the subarcualis rectus I. When it emerges from the muscle, the nerve is the ramus lingualis. It lies in the space between the ceratohyal and the first ceratobranchial, lying in loose connective tissue. It follows the first ceratobranchial to its juncture with the basibranchial, and then follows the first ceratobranchial, lying in loose connective tissue. It follows the first ceratobranchial to its juncture with the basibranchial, and then follows along the lateral margin of the basibranchial, within the tongue sheath, to the radii (second radii in *Salamandra*), where it rises sharply and enters the tongue pad proper. Francis ('34) described a small ganglion along the course of this ramus, but we

have not seen such a ganglion in any of the plethodontids we have studied.

COMPARATIVE ANATOMY

Theoretical considerations and predictions

Earlier publications in this series (Lombard and Wake, '76, '77) utilized a deductive methodology (see Dullemeijer, '80) that we will employ. Our approach is to start with the assumption that nonprojectile tongues are primitive in urodeles in general, and that the condition in *Plethodon* represents the point of departure for the evolution of projectile tongues in plethodontids (see Wake, '66, for a defense of these assumptions). We review the apparent morphological requisites of tongue projection in plethodontids, and then state our hypotheses in the form of predictions for deviations in patterns of peripheral nerve distribution during the evolution of tongue projection. This theoretical treatment will be the basis for the organization of our comparative analyses in the following section.

A major assumption is that patterns of nerve supply are conservative in evolution. In particular, we assume that nerve-muscle relationships are established very early in ontogeny, and remain stable. Further, we assume that during phylogeny these relationships are stable. Thus, muscle homologies are revealed in part by patterns of innervation.

Certain functional necessities are translated into morphological modifications as tongue-projection mechanisms evolve. The most important of these involves the attainment of tongue freedom. Because the entire articulated hyobranchial apparatus is projected in plethodontids, freedom of the tongue pad at its anterior point of attachment to the floor of the mouth is essential. The anterior attachment is strong primitively, for the stout genioglossus muscle extends from the region of the mandibular symphysis into the tongue pad. This muscle is short primitively, and one of the first morphological indications of incipient tongue projection is modification of the genioglossus (Wake, '66; Lombard and Wake, '77). All six of the tongue projectile modes of Lombard and Wake ('77) show modification of this muscle, with complete loss characterizing two modes (*Hydromantes* and supergenus *Bolitoglossa*). The muscle is absent in most species representing a third mode (*Eurycea* group). In the remaining spe-

cies that use the third mode, the muscle at most is reduced to a few poorly differentiated fibers (*Stereochilus*, *Typhlotriton*). The genioglossus of species that use the remaining three modes of tongue projection is moderately (*Hemidactylium*, *Ensatina*) to greatly (*Batrachoseps*) elongated.

When the genioglossus muscle is lost, the possibility of total tongue freedom exists, and the tongue in such genera as *Hydromantes*, *Bolitoglossa*, and *Eurycea* is attached to the body of the salamander only by the contents of the tongue sheath and the tissue of the sheath itself. In such species, a strongly differentiated stalk is evident in the projected tongue. But loss of the genioglossus, while necessary, is not sufficient to cause the loss of the anterior attachment, for *Typhlotriton* and *Stereochilus* have rudimentary genioglossus muscles at best but still retain a mucosal attachment of the tongue pad to the anterior floor of the mouth.

As anterior freedom and associated projectility are achieved, several modifications of the skeleton and musculature occur. For increased projectility, increased length of certain elements is necessary. The epibranchial always becomes elongated, as does that muscle responsible for projecting it, the subarcualis rectus. Further, this pinnately fibered muscle becomes increasingly complex and is formed into a multilayered bulb. The muscles that return the tongue to the mouth also become elongated. In the most extremely specialized species some of these muscles are lost, and the remaining externally elongated muscles are coiled in the floor of the mouth when at rest. The pad becomes compact, facilitating maneuverability. As the tongue becomes increasingly projectile, separation of once intimately associated muscles derived from the same embryological origin and having the same innervation, becomes great.

The projectile tongue retains a need for nerve supply. The tongue pad has many sensory endings, and even in the most specialized tongue projectors, it has at least one muscle and as many as three muscles. On embryological and phylogenetic grounds, innervation by the ramus lingualis of the glossopharyngeus (sensory) and ramus hypoglossus (motor) is expected, so long as sensation and pad musculature remain.

We predict that the ramus lingualis of the glossopharyngeus will be the more conservative of the two major nerves during evolution of tongue projection. The main trunk of the

glossopharyngeus is a mixed motor and sensory nerve, and the motor function is very important for tongue projection, because the supply is to the subarcualis rectus I. This is a complex muscle that has both a posterior bulblike wrapping around the epibranchial and an anterior straplike section. The nerve sends a large branch to the bulb, and then continues anteromedially to supply the anterior section. The ramus emerges from the medial border of the muscle and from this position it has a convenient route to the tongue pad, through a space along the dorsal border of the first ceratobranchial. It follows the ceratobranchial to the tongue sheath, which it enters. From this point the ramus extends along the lateral margin of the basibranchial to the radius, and then enters the tongue pad. We hypothesize no deviation from this direct route, but the nerve must become long in order to avoid being stretched as projection occurs. Coiling of this elongated nerve must occur, and the place most appropriate for such coiling would be the space between the medial border of the anterior part of the subarcualis rectus I and the tongue sheath, along the dorsal border of the first ceratobranchial. This space exists because of the requirement for contraction of the hyobranchial apparatus as it folds to enter the sheath during projection, and for expansion as it returns to the mouth. With increasing projectility the amount of coiling will increase proportionately, and it should fill the triangular space between the two ceratobranchials and the posterior part of the basibranchial. We expect the nerve to be anchored somewhere along the ceratobranchial or basibranchial in order to avoid complications with coiling and uncoiling during projection and retraction.

We expect the ramus hypoglossus to be less conservative than the ramus lingualis, for this entirely motor nerve must not only retain its historical contact with the muscles of the anterior floor of the mouth, but must also reach the muscles of the tongue tip and be able to travel with the tip during projection and retraction. Accordingly, the nerve must effectively bifurcate as the tongue gains freedom. The point of bifurcation is primitively at the extreme anterior end of the floor of the mouth, where the ramus hypoglossus branches three ways to serve first the geniohyoideus lateralis, then the genioglossus, and finally, by means of a posterodorsally directed branch, the hyoglossus and related

musculature in the tongue tip. The bifurcation that concerns us is the separation of the ramus to the tongue pad from the ramus hypoglossus proper. We predict that as the tongue gains projectility the bifurcation point should migrate posteriorly, at least as far posteriorly as the start of the tongue sheath, but even more posteriorly as coiling is required. We make this prediction for mechanical reasons, based on our assumption of the advantage of maximal simplicity in the highly mobile tongue. We think that an anterior bifurcation with a long, posteriorly oriented ramus extending at least as far back as the first ceratobranchial is less mechanically efficient than the alternative arrangement.

The hypoglossus, like the ramus lingualis, must have sufficient length to avoid stretching during projection. In relatively generalized species, in which most projection of the tongue is accomplished by flipping the pad out over the anterior margin of the lower jaw, we expect the nerve to be coiled in the pad itself, or in the tissue joining the pad to the front of the mouth, for we presume that the bifurcation point will be far forward and that the nerve supply to the muscles other than the tongue pad should not involve any increased length. As anterior tongue freedom is achieved, and with increasing projectility, we expect to see increased coiling between the bifurcation point and the tongue tip. But we expect such coiling to be in the tongue pad only if the pad shows considerable independent movement relative to the hyobranchial skeleton. If such is not the case, we predict that the nerve should be tightly bound to the skeleton, probably the basibranchial, so that the projectile is a maximally simplified linear unit. Thus we expect to see

coiling behind the basibranchial, in the space between it and the pericardium where there is loose connective tissue. In those species with the greatest tongue projectile capabilities, the urohyal is lost and the musculature associated with it is either lost or simplified, so that space for such coiling is available. The coiling is expected to be concentrated in one area, rather than being spread along the entire length of the nerve, for reasons of maximal mechanical efficiency and simplicity. Further, the coiling of the ramus hypoglossus should be well separated from that of the ramus lingualis, the former occupying a ventral and the latter a dorsal position, reasoning from their primitive patterns of orientation.

RESULTS

We have relatively complete information for four groups and we first present this, together with reconstructions based on serial histological sections, dissections, and the results of HRP experiments. Then we will present the information available concerning other plethodontid species.

Near-brain patterns are so similar in the species examined that we choose not to present a detailed comparative analysis at this time. The glossopharyngeus and the vagus share a large common ganglion, and the glossopharyngeus has a topological position on leaving the brain that is in direct line with the rootlets of the vagus, which usually consists of three major and some minor rootlets. Several rami issue from the ganglion. The most anterior one extends directly to the anterior border of the wound portion of the subarcualis rectus I, and there it splits into anterior and posterior rami. The anterior ramus give rise to the ramus lingualis. The

Abbreviations

Cartilaginous elements are stippled and indicated by uppercase letters, and nerves are indicated by lowercase abbreviations.

BB, basibranchial
br, basiradialis
CB 1, ceratobranchial 1
CB 2, ceratobranchial 2
EB, epibranchial
gg, genioglossus
ghl, geniohyoideus lateralis
ghm, geniohyoideus medialis
g. IX-X, ganglion of glossopharyngeal and vagus nerves
hg, hyoglossus
ir, interradians
R, radius

rep, rectus cervicis profundus
rcs, rectus cervicis superficialis
r. hyp., ramus hypoglossus
r, ramus lingualis
ling.,
sar, subarcualis rectus
sp, suprapeduncularis
sp. 1, spinal nerve 1
sp. 2, spinal nerve 2
*, bifurcation point (separation of lingual branch of ramus hypoglossus from branches to musculature of anterior floor of mouth; see text)
1, interruption of ramus hypoglossus
2, interruption of ramus lingualis and point where ramus hypoglossus joins ramus lingualis

main ramus of the glossopharyngeus is convoluted between the ganglion and the muscle.

Of the other branches of the vagus-glossopharyngeus complex, only one is of immediate concern to us. This is a ramus that arises from the ganglion immediately in front of the truncus intestino-accessorius and extends to the middle to hind part of the wound portion of the subarcualis rectus I.

The first spinal nerve arises from several rootlets and extends forward as the main or even exclusive part of the ramus hypoglossus. It is joined by a small branch of the second spinal nerve near the borders of the pharynx at some distance from the vertebral column. This generally has been considered to be a communicating ramus between the two nerves, but we suspect that it contains only fibers of the second spinal nerve. A branch of the ramus serves the rectus cervicis superficialis and rectus profundus muscles, as well as the hebstoeypsiloideus. The small section between this branch and the first spinal is probably only sensory, and the cutaneous fibers of the ramus hypoglossus are given off shortly after the ramus is formed by the merger of elements of the first two spinal nerves. Shortly after the ramus hypoglossus is formed, at the level of the carotid body, a branch extends to the rectus cervicis profundus.

The second spinal nerve has both dorsal and ventral roots and a ganglion. In addition to the components of the nerve mentioned above, a branch of the main ramus that arises near the point of origin of the communicating ramus serves the second and third segments of the rectus cervicis superficialis and the omohyoideus.

Distal patterns of innervation differ considerably among the species studied, and the following comments are organized by group, and with respect to our predictions.

Plethodon cinereus (Fig. 1)

This species was used as the point of departure for the entire study, and a general description was presented earlier.

The glossopharyngeus has a generalized course. The ramus lingualis is coiled and convoluted almost from its point of origin, which we interpret to be the first major branching of the main ramus of the glossopharyngeus. The coiling is initially dorsal to the distal end of the second ceratobranchial, but then moves to the dorsal surface of the first cera-

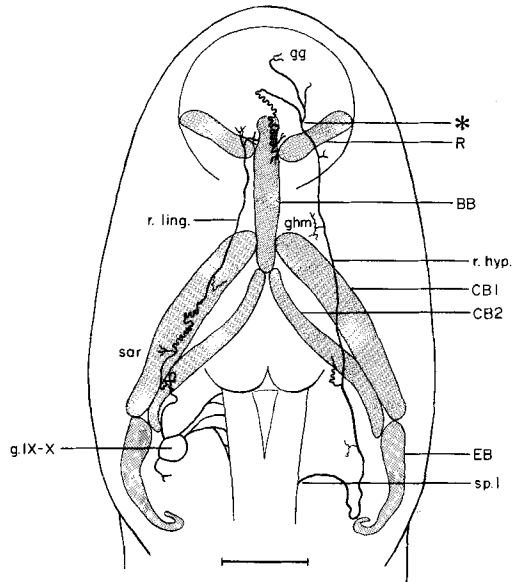


Fig. 1. General pattern of innervation of the tongue and associated musculature in *Plethodon cinereus*. Reconstruction from serial sections (stained by Palmgren method), whole mounts of specimens treated using horse-radish peroxidase technique, and dissections of freshly sacrificed specimens. Cartilaginous parts of the hyobranchial apparatus are stippled. Portions of the brain stem and tongue pad are outlined. Scale bar = 1 mm. See list of abbreviations.

tobranchial. There is no well-defined tongue sheath in *Plethodon*, and a short distance behind the basibranchial, the ramus lingualis straightens and extends along the lateral border of the rectus cervicis profundus, dorsolateral to the basibranchial, as far as the tongue pad. Within the tongue pad the ramus extends to the posterior border of the radius, where it bifurcates and, at the anterior border of the radius, turns dorsally to radiate into the surface of the tongue pad.

The ramus hypoglossus extends anteriorly from the region of the carotid body in a ventral position, below the distal third of the second ceratobranchial and the middle of the first ceratobranchial. It gives off branches along the way to the geniohyoideus medialis and suprapeduncularis. At the level of the radius a lateral branch extends to the geniohyoideus lateralis. Shortly thereafter, at the level of the anterior tip of the basibranchial, the nerve bifurcates, with the lateral branch going to the genioglossus. The medial branch extends dorsally and anteriorly as well as medially to the point in the extreme

anterior part of the mouth where the tips of the ceratohyals overlap. The ramus proceeds dorsally through the cleft between the ceratohyals, and into the tongue pad. It then immediately turns posteriorly and follows a path 180° away from the previous direction, to innervate the tongue pad muscles at the level of the radius. At the point where the ramus passes between the ceratohyal tips it becomes strongly convoluted, and remains so as far as the radius.

Aneides lugubris

This robust species has a tongue with a large pad that is attached by a strong connection to the anterior part of the mouth. There is a large, stout genioglossus. The pattern of innervation shows no significant deviation from that reported for *Plethodon cinereus* (Fig. 1). The ramus lingualis follows the dorsal margin of the first ceratobranchial until the rectus cervicis profundus muscle is reached. It then follows the dorsolateral margin of that muscle to the tongue pad. The ramus is convoluted to a modest extent along the ceratobranchial.

The ramus hypoglossus is a large trunk that extends to the anterior part of the mouth before bifurcating in a position well in advance of the tip of the basibranchial. The ramus that proceeds into the tongue pad is convoluted between the bifurcation with the main ramus, which serves the genioglossus, and the contact with the tongue pad musculature.

Ensatina eschscholtzii

This species has a tongue that is attached at the front of the floor of the mouth, but the attachment is rather loose, and there is a modest projectility of the tongue. The genioglossus is somewhat elongated and more flexible than in *Aneides*. The ramus lingualis of the glossopharyngeus is similar in position to that in *Plethodon* and *Aneides*, but differs in having considerably more convolution dorsal to the ceratobranchials.

The ramus hypoglossus is distinct from that of *Aneides* and *Plethodon* (cf. Fig. 1). The bifurcation point is far posterior, and the ramus does not enter the tongue pad from an anterior direction. The bifurcation point is approximately at the level where the first and second ceratobranchials come into contact, just in front of the epibranchial. The ramus to the tongue is immediately convoluted. It proceeds anteriorly, paralleling the main ramus, to a

point a little anterior to the posterior tip of the basibranchial. It then reverses and proceeds posteriorly as a loop that extends beyond the bifurcation point a short distance. Then the ramus again reverses and, maintaining its convolution, it extends anteromedially, roughly paralleling the inner margin of the first ceratobranchial. It enters a rather simple tongue sheath at the level of the anterior end of the first ceratobranchial, and as it passes under that element its convolutions end. The ramus then follows the ventrolateral surface of the basibranchial to the tongue pad, keeping entirely separate from the more dorsally oriented branch of the glossopharyngeal.

The main ramus of the hypoglossus remains in the floor of the mouth and extends far forward to the genioglossus, giving off a branch to the geniohyoideus lateralis at the level of the anterior end of the first ceratobranchial.

Batrachoseps attenuatus (Fig. 2)

This is a species that retains an anterior attachment of the tongue to the floor of the mouth, but this attachment is very loose, and the tongue has substantial projectile capabilities.

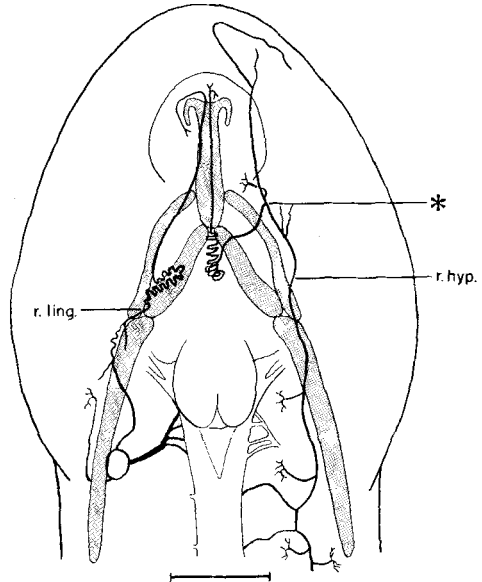


Fig. 2. General pattern of innervation of the tongue and associated musculature in *Batrachoseps attenuatus*. See Figure 1 for details. Scale bar = 1 mm.

The ramus lingualis is strongly convoluted from its origin, and it extends forward along the dorsal surface of the first ceratobranchial. The rectus cervicis profundus muscle moves from its ventral position behind the ceratobranchials to a dorsal position in front of them by passing through the space between the two cartilages. The convoluted ramus lingualis follows the dorsal border of the muscle as it rises into the space between the ceratobranchials and forms a long loop, convoluted along its entire course. The anterior-most end of this loop lies a little posterior to the posterior tip of the basibranchial. The loop then proceeds posteriorly, and at a point equivalent to the midpoint of the second ceratobranchial the ramus reverses direction and comes into close proximity with the dorsolateral border of the rectus cervicis profundus. In a short distance the convolution ceases and the ramus proceeds into the sheath of the tongue and continues to the region of the radius, in front of which it extends dorsally into the tongue pad.

The ramus hypoglossus is bifurcated far posteriorly, at the level of the attachment of the first ceratobranchial to the basibranchial. The ramus to the tongue then proceeds anteromedially at about a 45° angle to a point a little in front of the anterior end of the first ceratobranchial, and then turns sharply and becomes oriented directly posterior. When this ramus reaches the border of the ceratobranchial it again changes direction, moving posteromedially at about a 45° angle and crossing the anterior third of both ceratobranchials.

Immediately upon reaching the posteromedial border of the second ceratobranchial, the nerve proceeds almost directly posterior and becomes highly convoluted. This convoluted section forms a long loop extending near the midline back to the level of the anterior end of the epibranchial and then forward to the posterior end of the basibranchial. The convolution continues along the under surface of the basibranchial, but diminishes and becomes essentially straight in the main part of the tongue sheath, by the point of attachment of the first ceratobranchial, at which point the ramus rotates around the end of the cartilage and moves posteriorly to supply the muscles of the tongue pad.

The other portion of the bifurcated main ramus remains in its generalized position in the floor of the mouth. It gives off branches

to the suprapeduncularis, the geniohyoideus medialis, and the geniohyoideus lateralis muscles, and continues almost to the lower jaw, at which point it moves posterolaterally, paralleling the inner border of the mandible. The nerve at this point is traveling along the outer border of the elongated genioglossus. The nerve enters the genioglossus in the posterior part of the muscle.

Pseudoeurycea cephalica and P. leprosa (Fig. 3)

These two species display some minor differences, but overall they are so similar that we treat them together. Both have tongues that are entirely free anteriorly.

The ramus lingualis is strongly convoluted from its origin until well into the tongue sheath. In addition, there is at least one large loop in the convoluted ramus, in a position generally similar to that in *Batrachoseps*, that is, in the area posterodorsal to the rectus cervicis profundus where that muscle rises to pass between the two ceratobranchials. The ramus is somewhat posterior and medial to the position in *Batrachoseps*. Its convolutions start at the anterior end of the epibranchial,

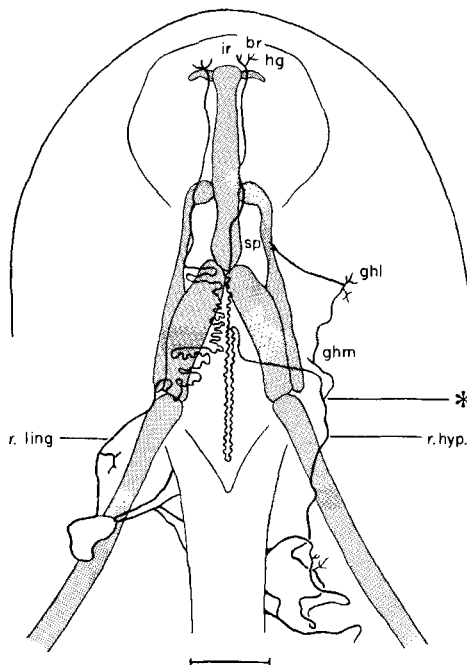


Fig. 3. General pattern of innervation of the tongue and associated musculature in *Pseudoeurycea leprosa*. See Figure 1 for details. Scale bar = 1 mm.

and the ramus proceeds anteriorly, with mainly a lateral loop, in the area above and even a little posterior to the second rather than the first ceratobranchial. In these species the distance across the ceratobranchials is rather narrow, and there is not so much space between these elements as in *Batrachoseps*. The ramus emerges from the main loop to proceed anteriorly almost along the midline, remaining highly convoluted as the sheath is reached, still a good distance behind the basibranchial. At the posterior border of the basibranchial the ramus, now in the tongue sheath which extends far posteriorly in *Pseudoeurycea*, again displays a moderately long lateral loop before finally moving laterally to the position of the first ceratobranchial. At this point, just in front of the midpoint of the ceratobranchial, it becomes anteriorly directed and follows the first ceratobranchial to the anterior tip of that element. The ramus now lies along the dorsolateral border of the rectus cervicis profundus and it follows that muscle forward. However, when the muscle rotates 180° upon entering the tongue and the ramus continues its anterior course, and finally rotates dorsally into the tongue at the anterior margin of the radius.

The ramus hypoglossus bifurcates very far posteriorly, just a little anterior to the anterior end of the epibranchial, or as far anterior as the midpoint of the second ceratobranchial. In *P. cephalica* the ramus to the tongue describes a tight arc, extending as far forward as the posterior end of the basibranchial and then moving posteromedially across both ceratobranchials to the midline, where it forms a long loop right on the midline. In *P. leprosa* the ramus proceeds almost directly medial from the point of bifurcation to the midline, and then forms a very long loop immediately on the midline. In both species the ramus is highly convoluted along the entire course of the loop, from the point at which the ramus approaches the midline to a position far inside the sheath of the tongue at a level equivalent to approximately the anterior one third of the first ceratobranchial and a little anterior to the posterior tip of the basibranchial. The loop is very extensive and lies in a dorso-ventral as well as an anterior-posterior plane. The loop lies in the space between the end of the basibranchial and the aortic arches.

When the convolution of the ramus to the tongue ends, the nerve lies close to the ventral surface of the basibranchial, to which it appears bound, as far anterior as the anterior end of the first basibranchial. Then it moves a little laterally and follows the rectus cervicis profundus forward to the tongue pad. The nerve rotates sharply dorsally around the anterior end of the radius to enter the pad.

The remaining branch of the main bifurcated ramus hypoglossus continues anteriorly through the floor of the mouth, progressively giving off branches to the genioidius medialis and the suprapenduncularis before ramifying in the geniophyoideus lateralis and terminating as a discrete ramus far behind the front of the mouth, for there is no genioglossus muscle in this genus.

Bolitoglossa rufescens

This species has a fully projectile tongue that is free from an anterior attachment. There is no genioglossus.

The tongue of this species is similar to but more projectile than that of *Pseudoeurycea* (Fig. 3). The ramus lingualis of the glosso-pharyngeus and the branch of the hypoglossus that serves the tongue pad remain separated for their entire length, although they travel side by side along the anterior part of the basibranchial. The ramus lingualis is strongly convoluted dorsal to the ceratobranchials, but is relatively straight after it is in the anterior part of the sheath, but as in other species the nerve is strongly convoluted in the folded-up base of the sheath. At the tongue tip the ramus lingualis enters the pad first, and the branch of the hypoglossus proceeds under the radius and then turns sharply up around the end of the radius into the pad.

Hydromantes italicus (Fig. 4)

This species has a tongue that is entirely free anteriorly, and has the greatest tongue projection ability among the species being compared. The arrangement of the hyobranchial apparatus at rest is rather different from that of the other species. In *Pseudoeurycea* the distance across the ceratobranchials is very narrow, but in *Hydromantes* the skeleton is spread out, and the distance across the ceratobranchials is great. The anterior end of the first ceratobranchial is located far posteriorly along the basibranchial,

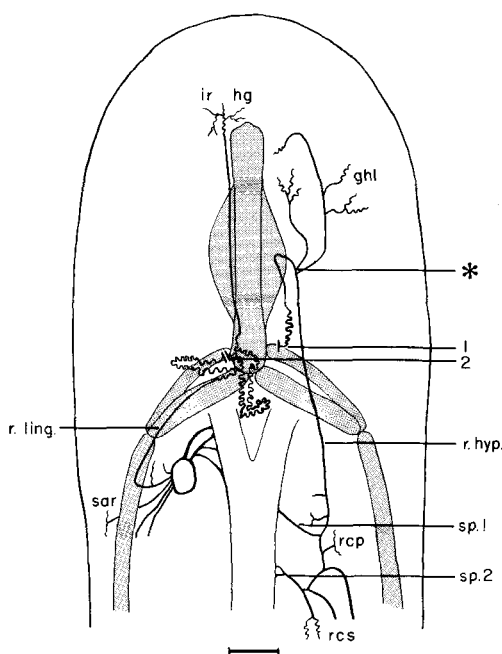


Fig. 4. General pattern of innervation of the tongue and associated musculature in *Hydromantes italicus*. See Figure 1 for details. In *Hydromantes*, ramus lingualis of nerve IX and ramus hypoglossus of the first spinal nerve join to form a common ramus. Ramus hypoglossus joins ramus lingualis at the points of interruption in the Figure (1 and 2), and from the juncture point forward the common ramus is illustrated on the left side of the Figure only. Scale bar = 1 mm.

in fact, near its posterior end (Fig. 4). The basibranchial is thus effectively much longer than in other species.

The ramus lingualis of the glossopharyngeus occupies the expected position. It emerges from the subarcualis rectus and extends medially, dorsal to the second ceratobranchial. Soon it becomes highly convoluted, and this convoluted segment is directed first medially and then loops laterally extending over the dorsal surface of the anterior portion of the first ceratobranchial, and again loops medially, finally merging with the lingual branch of the ramus hypoglossus at the level of the anterior end of the first ceratobranchial. The common trunk will be described below. The looped and heavy corrugated portion of the ramus lingualis lies above and behind the rectus cervicis profundus muscle where it rises to pass between the ceratobranchials.

The ramus hypoglossus bifurcates fairly far forward, at the level of the anterior end of the ceratobranchial or a little further forward. The branch to the tongue then continues anteriorly about to the midpoint of the basibranchial along the inner (medial) margin of the geniohyoideus medialis. At this point the nerve reverses course very sharply, and becomes directed posteromedially. It now becomes slightly convoluted, and the convolution increases modestly as the nerve passes through the anterior end of the first ceratobranchial and moves dorsally. It now joins the ramus lingualis of the glossopharyngeus to form a common trunk.

The common trunk of the lingual part of the hypoglossus and the ramus lingualis of the glossopharyngeus is highly convoluted almost from its origin, lateral to the anterior part of the first ceratobranchial. The convoluted segment proceeds posteriorly in a long and complex loop which occupies the space between the end of the basibranchial and the aortic arches, bounded laterally by the second ceratobranchials. The loop initially is far lateral, but it moves medially rather abruptly and then, after more convolution in a dorsal-ventral plane, extends anteriorly near the midline and enters the sheath of the tongue. Initially the convolutions continue, but again at the level of the anterior end of the first ceratobranchial the common trunk comes to lie in proximity to and along the dorsolateral margin of the rectus cervicis profundus, which it follows in a simple and direct way to the tongue pad. This common trunk shows no subdivision until the pad itself is reached, at which point, near the end of the expanded part of the basibranchial, separate branches rotate dorsally, well in front of the rectus cervicis profundus, to enter the tongue pad.

The remaining branch of the bifurcated ramus hypoglossus remains in the floor of the mouth. It gives off branches to the geniohyoideus medialis, the suprapeduncularis, and finally ramifies into the geniohyoideus lateralis. There is no genioglossus muscle, and hence the nerve terminates as a ramus rather far posteriorly.

Pseudotriton ruber and P. montanus

These species have fully projectile tongues that are free of anterior attachments. There is no genioglossus muscle. Distance of projec-

tion of the tongue is relatively short, judging from our manipulation of anesthetized specimens.

The arrangement of the ramus lingualis is similar to that of the other tongue-projecting salamanders. The convoluted and folded section lies primarily above the first ceratobranchial, and there is a large loop lying over the posterior third of the element that extends back to overlap the second ceratobranchial. Once out of the loop, the ramus straightens and proceeds anteriorly into the sheath, following first the first ceratobranchial and then the dorsolateral border of the rectus cervicis profundus.

The ramus hypoglossus differs from that of other species with fully projectile tongues. The bifurcation is located far anteriorly, in front of the anterior tip of the basibranchial. The nerve is convoluted immediately after the bifurcation point, and it arcs anteriomedially, reaching the anterior tip of the ceratohyal before proceeding posterodorsally. It then travels posteriorly to the mid-point of the articulation of the first ceratobranchial with the basibranchial, where in some specimens it then loops anteriorly and enters the tongue sheath. In other specimens the ramus extends back to the posterior tip of the basibranchial before looping anteriorly. Its convolution continues, even inside the tongue sheath. In this species the posterior border of the sheath is rather far forward, at the point where the nerve loops anteriorly. The nerve then continues to the tongue tip and remains separated from the ramus lingualis.

The nerve supply to the geniohyoideus medialis and suprapeduncularis leaves the ramus hypoglossus well posterior to the bifurcation point, and there are also apparently some fibers to the geniohyoideus medialis prior to the bifurcation. The branch of the ramus that remains in the floor of the mouth following the bifurcation quickly breaks up into small branches that serve the geniohyoideus lateralis.

Eurycea bislineata

This species has a fully projectile tongue that is free of anterior attachments. There is no genioglossus muscle. The general structure of the tongue and associated features is similar to that of the species of *Pseudotriton*, but the tongue is capable of substantially greater distance of projection.

The ramus lingualis has a course similar to that of *Pseudotriton*. It is at least as con-

volute and folded as in the species of that genus.

The ramus hypoglossus also has an arrangement that is similar to that encountered in *Pseudotriton*. The bifurcation is located far anterior, near the anterior tip of the ceratohyal. The main ramus of the nerve is much more prominent than the branch to the geniohyoideus lateralis. The main ramus extends forward for some distance anterior to the point of bifurcation. It then loops sharply posteriorly and medially, and a little dorsally, finally running almost directly posterior. As it reaches the level of the first ceratobranchial, convolutions begin, and these become increasingly great posteriorly. The ramus extends well posterior to the tip of the basibranchial, where it lies in a loose, convoluted coil just in front of the large urohyal. Evidently the ramus is more convoluted and looped, and is therefore relatively longer, than in *Pseudotriton*. In other respects the species of these two genera that we have examined are rather similar.

Hemidactylium scutatum

The tongue in this species is attached at the front to the floor of the mouth, but, as in *Ensatina*, the somewhat elongated genioglossus muscles result in a looser attachment than in such genera as *Plethodon* and *Desmognathus* (Lombard and Wake, '77).

The position of the ramus lingualis is very similar to that in *Pseudotriton* and *Eurycea*, and the ramus lingualis is in the apparently normal position for plethodontids.

The ramus hypoglossus has a bifurcation that is located far anteriorly, near the tip of the ceratohyal, and the branch to the genioglossus proceeds laterally toward the mandible. Prior to the bifurcation the ramus is somewhat irregular in course, and after the bifurcation the ramus becomes convoluted and it loops sharply anteromedially and then posteriorly. As it nears the basibranchial the ramus becomes greatly coiled, but the loops of the coil are very short. The ramus extends to a point in front of the anterior tip of the first ceratobranchial and then proceeds anteriorly again and enters the tongue pad. The coiled loop is much shorter than in either *Pseudotriton* or *Eurycea*.

DISCUSSION

Above we presented several predictions, based on theoretical arguments, concerning

expected evolutionary pathways for routes of peripheral nerves associated with the tongue during the evolution of projectility. The most conservative and obvious of these predictions is that the ramus lingualis of the glossopharyngeus nerve and the ramus hypoglossus of the first spinal nerve will maintain their ancestral association with the tongue and related structures, and such is the case.

We predicted that the ramus lingualis would be the more conservative of the two major nerves, and it is. Although the major muscle served by this nerve, the subarcualis rectus I, undergoes great changes in size and orientation, these are not changes that require any reorientation of the nerve route. Further, the convenient route to the tongue tip, along the ceratohyal and then with movement through a space to the tongue stalk, is retained by even the most morphologically specialized species. Increased tongue projectibility necessitates that the nerve be long in species with such specialization. At rest, this long nerve must be stored in a convenient place, and as expected, that place lies between the anterior exit of the nerve from the subarcualis rectus I and its entrance into the sheath that characterizes species with moderate to great tongue-projection capability (Lombard and Wake, '76; '77). The nerve in this region is both coiled and folded into a long loop, which as predicted lies dorsal to the rectus cervicis profundus in the triangular space lying between the ceratobranchials and the basibranchial. While we cannot quantify our observation, in such highly specialized tongue-projecting forms as *Hydromantes*, *Bolitoglossa*, *Pseudoeurycea*, and *Eurycea* the nerve is longer, more coiled, and has relatively larger loops than in less specialized species (Figs. 1-4).

We predicted that the ramus hypoglossus would be less conservative in its evolution than the ramus lingualis, and such is the case. There is substantial intergeneric variation in the peripheral distribution of the ramus hypoglossus. An effective bifurcation that separates a ramus to the tongue pad from the remainder of the ramus hypoglossus is found in all the species examined, but its position varies, as we predicted. Contrary to our predictions is the fact that there is not a simple functional relationship between the position of the bifurcation point and the degree of tongue freedom and projectility. Rather, there is a complex pattern that has both functional and phylogenetic components.

Two factors must be considered in evaluating the pattern of intergeneric variation in the bifurcation of the ramus hypoglossus. First is the fact that as tongue freedom is attained there is a great physical separation of the musculature of the tongue pad from that in the floor of the mouth, necessitating a modification of the ancestral condition. Second is the fact that as the tongue becomes increasingly capable of long-distance projection in different species, the nerve that serves the tongue tip musculature must become long. Some provision for storing this long nerve when the tongue is at rest is required, and some provision for assuring that the nerve efficiently moves out of and back into the storage position during tongue movement is required.

In *Salamandra salamandra* (Francis, '34; confirmed by our own preparations) the bifurcation occurs far forward, at the level of the genioglossus muscle. This position is also found in *Plethodon* and *Aneides*. Accordingly, on the basis of this out-group comparison, we consider this position to be the ancestral one for the family Plethodontidae.

We predicted that the point of bifurcation of the ramus hypoglossus would migrate posteriorly as species became increasingly capable of projecting their tongues. This prediction was based simply on functional considerations. In fact, we observed a posterior migration of this bifurcation in *Ensatina*, *Batrachoseps*, *Bolitoglossa*, *Pseudoeurycea*, and *Hydromantes*. These genera demonstrate increasing anterior tongue freedom in the order in which they are listed. The posterior migration of the bifurcation point occurs only in species with some degree of tongue freedom. However, in both *Pseudotriton* and *Eurycea*, genera with complete tongue freedom, in contrast to *Ensatina* and *Batrachoseps*, the bifurcation point is essentially in its ancestral condition (although the region is modified as a result of the loss of the genioglossus muscle, and of course its innervation). *Hemidactylium*, with its loosely attached tongue, contrasts with *Ensatina* and *Batrachoseps* in having an anterior bifurcation, and it is otherwise more similar to *Eurycea* and *Pseudotriton* than to any other genera.

Obviously evolution of tongue freedom is not sufficient to result in movement of the bifurcation point posteriorly. This part of the nervous system and the biomechanical parts of the tongue related to tongue freedom as

well as increased distance of projection are capable, in theory, of evolving independently. Because function alone is an insufficient predictor of nerve route pattern, we seek a possible explanation in the phylogenetic history of the groups.

Eurycea and *Pseudotriton* are members of the tribe Hemidactyliini. *Hemidactylum*, also a member of the Hemidactyliini, has a tongue that retains a well-developed, but somewhat elongated, genioglossus muscle (Lombard and Wake, '77). Wake ('66) argued that the presence of this only slightly modified tongue in the Hemidactyliini was evidence that highly projectile tongues had evolved independently in the Hemidactyliini (for example, in *Eurycea* and *Pseudotriton*, which are thought to have had a common, free-tongued ancestor) and in the Bolitoglossini. Our interpretation of the situation in *Eurycea* and *Pseudotriton* is that they have retained an ancestral bifurcation pattern (seen also in *Hemidactylum*). However, while they retain an ancestral trait in one part of the nerve distribution pattern, they have a derived pattern for providing increased length of the ramus hypoglossus that is not found in other species with tongue-projection capability. Further, although we did not consider their pattern *a priori* to be a likely one to evolve, it is very effective. The ramus makes a sharp reversal in its course at the anterior end of the ceratohyal. The position of this reversal, at a point just anterior to the bifurcation point, is far anterior and it is also a fixed point in relation to the movable parts of the tongue. Accordingly, the long section of the nerve that lies between the point where it reverses course at the anterior end of the tongue and the point which the nerve again reverses course to move anteriorly into the movable parts of the tongue accounts for a relatively large proportion of the storage of the nerve, which otherwise would have to be more coiled and folded than it is.

A possible explanation for this pattern may relate to the evolutionary history of the Hemidactyliini. *Hemidactylum* has a well developed genioglossus, and the ancestral pattern (such as occurs in *Plethodon* and *Aneides*) is for the bifurcation point to be beyond the point at which the nerve to the genioglossus leaves the ramus hypoglossus. Further, both *Stereochilus* and *Typhlotriton*, also members of the Hemidactyliini, retain remnants of a genioglossus (Lombard and Wake, '77). There is some question concerning the monophyletic status of the Hemidac-

tyliini; *Hemidactylum* is aberrant in several regards (Wake, '66; Wake and Lombard, '73). *Hemidactylum* has a similar bifurcation pattern as *Eurycea* and *Pseudotriton*, and we predict that such a pattern will be found in *Typhlotriton* and *Stereochilus*.

There are several reasons for thinking that *Ensatina* is a close relative of *Plethodon* and *Aneides* (Dunn, '26; Noble, '31; Wake, '66; Larson et al., '81). Because it has the derived bifurcation pattern, an implication of this finding is that the derived bifurcation pattern has evolved independently in the Plethodontini and the Bolitoglossini. This is one more feature associated with the acquisition of partial tongue freedom in *Ensatina*, and it is a further indication that tongue freedom has evolved multiply in the Plethodontidae (cf. Wake, '66; Lombard and Wake, '77).

We predicted that the ramus hypoglossus would be coiled and folded in the space that lies between the end of the basibranchial and the heart. In fact, in all species with some degree of tongue freedom the ramus hypoglossus shows both coiling along its length and folding, and in all instances the maximally coiled and folded region is near the posterior end of the basibranchial. However, in all instances the ramus also is coiled anterior to this point for at least a short distance. In *Eurycea* and *Pseudotriton* the urohyal is a very large structure and it is located far anteriorly, very near the posterior end of the basibranchial. Thus in these genera there is less space in this region than in other forms with some tongue freedom. This is a further complication in explaining the far anterior bifurcation pattern in these genera. In the Bolitoglossini there is no urohyal and there is a relatively large amount of space; in genera of this tribe the region of maximal coiling and folding of the ramus hypoglossus is in this spacious region.

In all genera that we examined there was at least some coiling of the ramus hypoglossus. The least amount was found in *Plethodon* and *Aneides*, groups with restricted projection, in which the coiling occurs in the anterior part of the tongue pad itself. In no other genera have we observed any coiling in the anterior part of the pad. Rather, all (except the somewhat aberrant *Hemidactylum*) show the maximal amount of coiling in the posterior end of the basibranchial. Once the nerve enters the tongue sheath it appears to be bound tightly to surrounding tissues and it is straight and uncoiled from this point (near the attachment of the first ceratobran-

chial to the basibranchial) all the way to the tongue tip.

Presence of a genioglossus muscle is not sufficient to constrain the bifurcation point from migrating posteriorly. In *Ensatina* a well-developed but elongated genioglossus is present, but the bifurcation point has migrated far posteriorly.

Perhaps our most unexpected discovery was the merger of the ramus lingualis and the ramus hypoglossus into a common trunk in *Hydromantes*. To our knowledge the complete merger of these two nerves, well distal to the brain, is a condition unique in the vertebrates. This seems to be an arrangement with functional significance. *Hydromantes* can project its tongue an appreciably greater distance than any other salamander, and it is more specialized in many features for tongue projection than is any other salamander (Lombard and Wake, '77; Wake, '82). Both the ramus lingualis and ramus hypoglossus must be coiled greatly in this genus. Following only modest amounts of coiling, the two nerves join to form a common trunk, which then is itself greatly coiled, twisted, and looped. The bilateral trunks come in intimate contact with each other, posterior to the basibranchial, and this merger is evidence that the greater amount of tongue projection in *Hydromantes* evolved after the nerve specialization evolved. This is one additional piece of evidence that argues in favor of the view that *Hydromantes* might have evolved its entirely free, highly projectile tongue independently of the other members of the Bolitoglossini in the supergenus *Bolitoglossa* (represented in this study by *Pseudoeurycea* and *Bolitoglossa*).

Within the subfamily Plethodontinae alone, fully projectile tongues are thought to have evolved at least two and probably three times (Wake, '66, '82). The results of the present investigation are concordant with this view, and support the idea of extensive parallel evolution in the family Plethodontidae. If one accepts for the sake of argument the monophyletic nature of the three tribes Hemidactyliini, Plethodontini, and Bolitoglossini, and the proposition that the nerve pattern seen in *Plethodon* and *Aneides* is close to the ancestral one, the situation can be summarized briefly. Within the Hemidactyliini, *Hemidactylium* has a tongue that retains a genioglossus muscle (an elongated one [Lombard and Wake, '77]) and is thus technically not freely projectable. However, the free-

tongued members of that tribe that have been examined share a unique pattern of peripheral nerve distribution, easily derived from the situations seen in *Plethodon* and *Hemidactylium*. Within the tribe Bolitoglossini the genus *Batrachoseps* also has a tongue that retains a genioglossus muscle (one that is even more elongated than in *Hemidactylium* [Piatt, '35; Lombard and Wake, '77]), and thus is technically not freely projectable. The other genera of this tribe are free-tongued. In the unlikely event that genioglossus muscles have re-evolved in one or both tribes, we are forced to conclude that free tongues have evolved at least twice in the family (Wake, '66; '82). The free-tongued bolitoglossines have a different nerve pattern than do the free-tongued hemidactyliines. Further, *Hydromantes*, a bolitoglossine, has a uniquely derived pattern of innervation, and because it is likely that it diverged from an ancestral stock that later gave rise to both *Batrachoseps* and the remaining group of bolitoglossine genera of the supergenus *Bolitoglossa* (Wake, '66; Elias and Wake, '83; Wake, unpublished data), it represents a third independent derivation of an entirely free and projectile tongue. Finally, in the genus *Ensatina* of the tribe Plethodontini a partially free (with an elongated genioglossus muscle) tongue that has a nerve pattern resembling that in *Batrachoseps* and the supergenus *Bolitoglossa* is found. Accordingly, we conclude that tongue projectibility in the subfamily Plethodontinae has evolved in total or extensive independence at least four (Hemidactyliini, *Ensatina*, *Batrachoseps*-supergenous *Bolitoglossa*, and *Hydromantes*), and perhaps as many as six times (separating *Hemidactylium* from the other hemidactyliines, and separating *Batrachoseps* from the supergenous *Bolitoglossa*). This conclusion and its implications will be more fully explored in future publications.

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LITERATURE CITED

- Bowers, M.A. (1900) Peripheral distribution of the cranial nerves of *Speleperpes bislineatus*. Proc. Am. Acad. Arts Sci. 36:179-193.
- Drüner, L. (1901) Studien zur Anatomie du Zungenbein-, Kiemenbogen- und Kehlkopfmuskeln der Urodelen. I. Theil. Zool. Jahrb. Abt. Anat. Ont. 15:435-622.
- Drüner, L. (1904) Studien zur Anatomie der Zungenbein-, Kiemenbogen- und Kehlkopfmuskeln der Urodelen. II. Theil. Zool. Jahrb. Abt. Anat. Ont. 19:361-690.
- Duillemeijer, P. (1980) Functional morphology and evolutionary biology. Acta Biotheor. (Leiden) 29:151-250.
- Dunn, E.R. (1926) The salamanders of the family Plethodontidae. Northampton, Massachusetts. Smith College Fiftieth Anniversary Publ.
- Edwards, J.L. (1976) Spinal nerves and their bearing on salamander phylogeny. J. Morphol. 148:305-328.
- Elias, P., and D.B. Wake (1983) *Nyctanolis pernix*, a new genus and species of plethodontid salamander from northwestern Guatemala and Chiapas, Mexico. In A. Rhodin and K. Miyata (eds): Advances in Herpetology and Evolutionary Biology. Cambridge, Massachusetts: Museum of Comparative Zoology, Harvard University, pp. 1-12.
- Fox, H. (1954) Development of the skull and associated structures in the Amphibia with special reference to the urodèles. Trans. Zool. Soc. London 28:21-304.
- Francis, E.B.T. (1934) The Anatomy of the Salamander. Oxford: Clarendon Press.
- Fritsch, B. (1981) The pattern of lateral line afferents in urodèles. Cell Tissue Res. 218:581-594.
- Hecht, M.K., and J.L. Edwards (1977) The methodology of phyletic inference above the species level. In M.K. Hecht, P.C. Goody, and B.M. Hecht (eds): Major patterns in Vertebrate Evolution. New York: Plenum Press, pp. 3-51.
- Jarvik, E. (1963) The composition of the intermandibular division of the head in fish and tetrapods and the diphyletic origin of the tetrapod tongue. K. Svenska Vetensk Akad. Handl. 9:1-74.
- Larson, A., D.B. Wake, L.R. Maxson, and R. Highton (1981) A molecular phylogenetic perspective on the origins of morphological novelties in the salamanders of the Tribe Plethodontini (Amphibia, Plethodontidae). Evolution 35:405-422.
- Lombard, R.E., and D.B. Wake (1976) Tongue evolution in lungless salamanders, Family Plethodontidae. I. Introduction, theory and a general model of dynamics. J. Morphol. 148:265-286.
- Lombard, R.E. and D.B. Wake (1977) Tongue evolution in the lungless salamanders, Family Plethodontidae. II. Function and evolutionary diversity. J. Morphol. 153:39-80.
- Magimel-Pelonnier, O.L. (1924) La Langue des Amphibiens (Anatomie et ontogenie comparees de la forme et des muscles). These, Fac. Sci. Paris, pp. 1-256.
- Noble, G.K. (1931) Biology of the Amphibia. New York: McGraw-Hill.
- Özeti, N., and D.B. Wake (1969) The morphology and evolution of the tongue and associated structures in salamanders and newts (family Salamandridae). Copeia 1969: 91-123.
- Piatt, J. (1935) A comparative study of the hyobranchial apparatus and throat musculature of the Plethodontidae. J. Morphol. 57:213-251.
- Piatt, J. (1938) Morphogenesis of the cranial muscles of *Amblystoma punctatum*. J. Morphol. 63:531-587.
- Piatt, J. (1940) Correct terminology in salamander myology. II. Transverse ventral throat musculature. Copeia 1940:9-14.
- Regal, P.J. (1966) Feeding specializations and the classification of terrestrial salamanders. Evolution 20:392-407.
- Roth, G. (1976) Experimental analysis of the prey catching behavior of *Hydromantes italicus* Dunn (Amphibia, Plethodontidae). J. Comp. Physiol. 109:47-58.
- Smith, L. (1920) The hyobranchial apparatus of *Speleperpes bislineatus*. J. Morphol. 33:527-583.
- Szamoylenko, E. (1904) Muskulatur, Innervation und Mechanismus der Schleuderzunge bei *Speleperpes fuscus*. Inaugural-Dissertation, Albert-Ludwigs-Universität, Freiburg im Breisgau.
- Thexton, A.J., D.B. Wake, and M.H. Wake (1977) Control of tongue function in the salamander *Bolitoglossa occidentalis*. Arch. Oral Biol. 22:361-366.
- Wake, D.B. (1966) Comparative osteology and evolution of the lungless salamanders, family Plethodontidae. Mem. So. Calif. Acad. Sci. 4:1-111.
- Wake, D.B. (1982) Functional and developmental constraints and opportunities in the evolution of feeding systems in urodèles. In D. Mossakowski and G. Roth (eds): Environmental Adaptation and Evolution (4th Bremen Symposium on Biological Systems Theory). G. Fischer, Stuttgart, pp. 51-66.
- Wake, D.B. and R. Lawson (1973) Developmental and adult morphology of the vertebral column in the plethodontid salamander *Eurycea bislineata*, with comments on vertebral evolution in the Amphibia. J. Morphol. 139:251-300.
- Wake, D.B., and R.E. Lombard (1971) Functional aspects of tongue projection mechanisms in plethodontid salamanders. Herp. Rev. 3:108.
- Wake, D.B., and R.E. Lombard (1973) Feeding mechanisms and the evolution of plethodontid salamanders. HISS News-Journal 1:64-65.