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The Evolution of Neural Circuits Controlling Feeding Behavior in Frogs

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Abstract

Our approach to understanding motor systems is a phylogenetic, 'outside-in' approach, the goal of which is to identify behavioral transitions during phylogenesis and elucidate their neurological basis. In this paper, we review the results of recent behavioral, biomechanical and neurological studies on frog feeding behavior. These studies show that highly protrusible tongues have evolved numerous times independently among frogs, and that the biomechanics and neuromuscular control of feeding behavior have been transformed repeatedly during frog evolution. Many of the independent lineages possess unique biomechanical mechanisms for protracting their tongues and unique neural mechanisms for coordinating feeding behavior. In frogs, there has been considerable evolution at the interface between reticular central pattern generators (CPGs) associated with feeding and sensory feedback circuits that modulate feeding motor output. In particular, the roles of hypoglossal and glossopharyngeal sensory feedback appear to have been relatively plastic in their evolution. Prey-type dependence of hypoglossal sensory feedback in *Rana* suggests that the interaction between descending visual control and sensory feedback also may be evolutionarily plastic. Comparative studies have found that motor systems sometimes evolve conservatively across morphological and behavioral transitions (i.e., the shoulder in birds) or, alternatively, they may be subject to considerably more evolutionary change than is reflected in morphological characteristics (i.e., feeding in cichlids). We hypothesize that the CPG circuits for feeding behavior in the reticular formation may evolve conservatively because they are highly integrated, multifunctional networks which cannot be optimized for one function without compromising others. In contrast, the interfaces between the CPG, sensory feedback and descending control should be less constrained. When changes in motor patterns occur during evolution, it is likely that sensory feedback or descending control may be involved.

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Introduction

Our approach to motor systems is an 'outside-in' approach, in which studies of behavioral phenomena are used to generate hypotheses about biomechanics and neural control. These hypotheses are then tested using the methods of functional morphology, comparative neuroanatomy and neurophysiology. Our approach is also a phylogenetic approach similar to that endorsed by Lauder [1986].

Morphological and neurological data are mapped onto a cladogram derived from other data. From these data, the ancestral or plesiomorphic state can be reconstructed, and evolutionary transitions from plesiomorphic to derived states can be identified. By identifying behavioral transitions within lineages, the behavioral, morphological, biomechanical and neurological changes associated with the acquisition of new behavior patterns during evolution can be elucidated.

All neural systems are derived from previously existing systems that have been modified to serve new functions [Northcutt, 1984; Dumont and Robertson, 1986; Cohen, 1988]. Thus, how neural circuits are modified during behavioral evolution is a fundamental question of neurobiology. Comparative behavioral and neurological studies are the only means to achieve three goals: (1) to elucidate the neural basis for the evolution of derived behavior patterns by comparing plesiomorphic vs. derived character states within lineages; (2) to elucidate the developmental basis of derived behavior patterns by comparing the ontogenies of plesiomorphic vs. derived character states within lineages; and (3) to derive general principles of neural evolution by repeated application of this program in distantly related lineages.

One general principle that appears to be emerging from comparative studies is that patterns of motor activity are often less variable, in both ontogeny and phylogeny, than musculoskeletal elements [Lauder and Shaffer, 1988; Wainwright et al., 1989]. Thus, across many behavioral transitions, the morphology of the musculoskeletal apparatus differs more than the pattern of motor activity. An example of conservative motor activity across a morphological and behavioral transition during ontogeny is the pattern of muscle activity during aquatic suction feeding in salamanders (Ambystoma tigrinum), which is similar before and after metamorphosis despite extensive remodeling of the hyobranchial apparatus [Lauder and Shaffer, 1988]. An example of conservative motor behavior across a morphological and behavioral transition during phylogeny is the pattern of activity of shoulder muscles during flight in birds, which is similar to the activity of the same muscles during locomotion in other tetrapods, despite the profound morphological and behavioral changes associated with the evolution of flight [Peters and Goslow, 1983; Goslow et al., 1989].

There are, of course, many cases in which motor patterns appear to be as evolutionarily plastic as morphology, or even more so [Gordon and Herring, 1987]. For example, some groups of African cichlid fishes exhibit little morphological differentiation among species but extremely diverse feeding behavior, as well as diverse patterns of motor activity [Liem, 1978, 1979]. In an elegant comparative study, Lauder [1983] also showed that the ability to feed on mollusks, which is a derived feature of sunfishes, was accompanied by the evolution of a novel pattern of motor activity during feeding.

A second general principle that has emerged from a number of morphological studies is the idea that highly integrated functional systems may evolve more slowly than systems that have a greater number of independent parts [Lauder and Liem, 1989; Roth and Wake, 1989]. In highly integrated systems, each component plays a role in multiple functions. Consequently, it is less likely that a given component can be optimized for a given function. The greater the independence of the components, the more specialized each component can become for a particular function. Duplication of parts, as in the jaw joint of mammal-like reptiles or in gene duplication, is a common mechanism of uncoupling [Lauder and Liem, 1989]. An example of the role of uncoupling in evolutionary diversification is the hyobranchial apparatus of salamanders, which primitively plays a role in both respiration and feeding. Through loss of lungs, the hyobranchial apparatus of plethodontid salamanders has been uncoupled from the breathing process and has become highly specialized for feeding [Wake, 1982].

Frog Feeding Behavior as a Model Motor System

We have chosen the feeding behavior of frogs as a model motor system for evolutionary studies because frogs exhibit a remarkable diversity of feeding behavior, and therefore provide a unique opportunity to study the evolution of motor control during behavioral diversification among closely related species. The goal of our research program is to identify evolutionary changes in the neuromuscular control of feeding behavior during phylogenesis, and to elucidate their anatomical and physiological basis.

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Evolution of Feeding Behavior in Frogs

Frogs are visual predators, and they must accurately recognize and precisely localize prey items in the visual field. Once the processes of prey recognition and localization have been carried out, a frog's brain must produce a motor response that is oriented in the correct direction at the correct distance, and the response must occur within a time period short enough to insure prey capture.

Frog feeding behavior is a good model system because the major brain centers involved in feeding movements have been identified. In the current hypothesis for motor control of frog feeding behavior (fig.1), incoming visual signals are sent from the retina to the optic tectum. Stimulus properties are analyzed within the tectum itself [Lettvin et al., 1959; Grüsser and Grüsser-Cornehls, 1976; Ewert, 1984, 1987; Roth, 1987], or in thalamic and isthmic nuclei and relayed back to the tectum [Grobstein and Comer, 1983; Ingle, 1983; Grobstein et al., 1985]. The optic tectum contains the cells of origin of the tractus tectobulbaris rectus and cruciatus [Weerasuriya and Ewert, 1981; Weerasuriya, 1983], which provide monosynaptic inhibitory and excitatory input to the ipsilateral reticular formation and polysynaptic input to the contralateral reticular formation [Matsushima et al., 1989]. Motor pattern generators in the reticular formation [Satou et al., 1984, 1985] provide input to the motor neurons of feeding muscles (fig.1).

The biomechanics of frog feeding behavior has been studied only in *Bufo marinus* [Emerson, 1977; Gans and Gorniak, 1982a, b] and *B. japonicus* [Matsushima et al., 1985]. In the presently accepted model of feeding biomechanics in *Bufo*, the anteriorly attached, stiffened tongue is rotated forward and flipped over the depressed mandibular symphysis by a muscular wedge-and-lever system [Gans and Gorniak, 1982a, b]. Muscle activity during feeding behavior also has been studied only in the genus *Bufo* [Gans and Gorniak, 1982a, b; Matsushima et al., 1985; Nishikawa and Gans, 1992].

Variation in the morphology of the feeding apparatus has been studied extensively in frogs [Magimel-Pelonnier, 1924; Emerson, 1976, 1985; Regal and Gans, 1976; Horton, 1982; Cannatella, 1985]. Previous studies identified four basic patterns of tongue morphology: (1) 'Discoglossoid' tongues are round and broadly attached to the floor of the mouth and are found in the least derived groups of anurans, i.e., *Ascaphus, Leiopelma, Bombina* and *Discoglossus*, and in some neobatrachians, including *Telmatobius* and *Cyclorana* [Horton, 1982; Regal and Gans, 1976]; (2) an anteriorly free, hydrostatic tongue is found in *Rhinophrynus dorsalis* [Trueb and Cannatella, 1982; Trueb and Gans, 1983]; (3) the aquatic pipid frogs (including
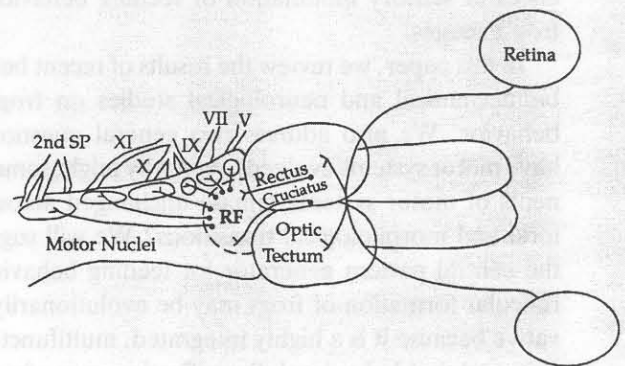

Fig. 1. Schematic diagram of a frog brain showing connections between the retina, the optic tectum, the reticular formation (RT) and the motor neurons that control feeding muscles. Retinal ganglion cells project from the retina to the optic tectum. The optic tectum projects to the reticular formation, via the tractus tectobulbaris rectus and cruciatus, and the reticular formation projects to brainstem and spinal motor neurons.

Xenopus laevis) are secondarily tongueless [Cannatella and Trueb, 1988], although they possess vestiges of tongue musculature [Horton, 1982]. They appear to be suction or ram feeders [Sokol, 1969; Avila and Frye, 1977]. (4) An anteriorly attached, muscular tongue with a free posterior flap is present in most other species (i.e., most pelobatoids and neobatrachians) [Regal and Gans, 1976]. The kinematics of feeding behavior has been studied previously in several species of frogs [*Ascaphus*, Larsen and Guthrie, 1975; *Xenopus*, Avila and Frye, 1977; *Hymenochirus*, Sokol, 1969; *Rana*, Gans, 1961, 1962; *Rana, Bufo, Kaloula, Pyxicephalus*, and *Dyscophus*, Emerson, 1985].

We have studied the biomechanics and neuromuscular control of frog feeding behavior using an approach that combines nerve transection studies with kinematic analyses of feeding behavior. This approach permits a test of the hypothesis that a given muscle is necessary or sufficient for the performance of a given component of motor behavior [Nishikawa and Roth, 1991]. A muscle is necessary for a given movement if denervation of that muscle alters the kinematics of the behavior or eliminates it entirely. A muscle is sufficient for a given movement if denervation of other relevant muscles has no effect on the behavior. If the transected nerve also contains sensory fibers, changes in movement may be caused by disruption of sensory feedback. While our initial efforts were directed toward using these techniques to identify differences in feeding biome-

chanics among frogs, we also have begun to study differences in sensory modulation of feeding behavior among frog lineages.

In this paper, we review the results of recent behavioral, biomechanical and neurological studies on frog feeding behavior. We also address two general questions: How have motor systems evolved? And why might some components of motor systems remain unchanged across behavioral and morphological transitions? We will suggest that the central pattern generator for feeding behavior in the reticular formation of frogs may be evolutionarily conservative because it is a highly integrated, multifunctional circuit, and that behavioral diversification may often involve evolution at the interface between CPGs and less integrated components, such as descending control from higher brain centers and sensory feedback [Cohen, 1992].

Materials and Methods

Kinematic and phylogenetic analyses, biomechanics, and analysis of neuromuscular control, proceeded as follows.

Kinematic Analysis

A Display Integration Technologies model DIT 660 high-speed, multi-framing video camera with Instrobe™ 90AS synchronized stroboscopic illumination was used to film the frogs in lateral view at 120–240 fields per second. Frogs were videotaped while feeding at room temperature (approximately 22–23 °C), using waxworms (*Galleria* sp.), earthworms (*Lumbricus* sp.), crickets (*Gryllus* sp.) or fruitflies (*Drosophila melanogaster*) as prey. Videotapes were analyzed with Peak Performance Technologies 2D motion analysis software. On each frame, the X, Y coordinates of the prey item, 10 points on the head of the frog, and a non-moving reference point were digitized from the video monitor [Nishikawa and Cannatella, 1991; Nishikawa and Roth, 1991].

Phylogenetic Analysis

Individual frogs representing 65 species, 46 genera and 13 families were studied. For the phylogenetic analysis, only the frame of maximum tongue protraction was digitized for each sequence. The following variables were calculated directly from the digitized points: (1) tongue reach = maximum distance tongue protrudes beyond the line formed by the tips of the upper and lower jaws; (2) jaw length = the distance from the jaw joint to the anterior tip of the upper jaw; and (3) tongue angle = angle subtended by the jaw joint and the tip of the tongue, with the tip of the lower jaw at the vertex. Relative tongue reach was calculated as tongue reach/jaw length [Cannatella et al., 1992]. Frogs were categorized as having protrusible or highly protrusible tongues on the basis of relative tongue length [Cannatella et al., 1992]. In our sample of 65 anuran species, relative tongue length exhibited a bimodal distribution with a gap between those species with relatively long tongues and those with relatively short tongues. The value of 65% represents the midpoint of the gap in the distribution. Thus, protrusible tongues are less than 65% of jaw length, whereas highly protrusible tongues are greater than 65% of jaw length.

A phylogenetic hypothesis for anurans was derived from Cannatella [1985] for basal groups and from Duellman and Trueb [1986], with some modifications, for neobatrachians [Cannatella et al., 1992]. The character states (protrusible vs. highly protrusible) were mapped onto the anuran phylogeny, and the minimum number of evolutionary changes in tongue length was computed [Cannatella et al., 1992].

Biomechanics

To study the biomechanics of frog feeding behavior, several individuals of selected species were filmed feeding under three conditions [Nishikawa and Roth, 1991; Deban and Nishikawa, 1990, 1992]: (1) before surgical transection of selected nerves; (2) after surgical transection of selected nerves; and (3) after control surgeries which were identical to surgical transection except that the nerve was exposed but not transected. For surgical transection, the frogs were anesthetized by immersion in 10% ethanol or 1% MS222 (tricaine methanesulfonate). Selected nerve branches were transected surgically near the point where they enter their targets. All nerves are both sensory and motor, except where otherwise noted. A 2 mm length of the nerve was excised to retard regeneration. Following recovery from anesthesia (1–2 h after surgery), the first feeding attempts of each frog were videotaped as above [Nishikawa and Roth, 1991].

The following kinematic variables were analyzed: (1) duration of approach = onset of forward head movement to maximum forward excursion; (2) duration of mouth opening = onset of mouth opening to maximum gape; (3) duration of mouth closing = maximum gape to completion of mouth closing; (4) duration of tongue protraction = onset of tongue protraction to maximum tongue protraction; (5) duration that tongue remains at target = prey contact to onset of tongue retraction; (6) duration of tongue retraction = onset of tongue retraction to completion of tongue retraction; (7) duration of feeding sequence = onset of forward head movement to completion of mouth closing; (8) duration of body recovery = maximum forward excursion to completion of mouth closing [Nishikawa and Roth, 1991].

The following additional variables were calculated directly from the digitized points: (1) minimum angle of mandibular bending = angle subtended by the jaw joint and the tip of the lower jaw with the midpoint of the lower jaw at the vertex; (2) maximum gape angle = angle subtended by the tips of the upper and lower jaws with the jaw joint at the vertex; (3) maximum absolute gape = distance between tips of upper and lower jaws; (4) distance to prey = distance between tip of snout and prey before onset of forward head movement; (5) lunge distance = distance between position of tip of snout at rest and at maximum forward excursion; (6) overshoot distance = distance between tip of snout at prey contact and tip of snout at maximum forward excursion; (7) maximum tongue reach = maximum distance tongue protrudes beyond the line formed by the tips of the upper and lower jaws; (8) maximum tongue height = maximum distance between the top of the tongue pad and the lower jaw; (9) maximum angular velocity of mouth opening; and (10) maximum angular velocity of mouth closing. Kinematic variables were compared before and after denervation using paired t-tests [Nishikawa and Roth, 1991].

Neuromuscular Control of Feeding Behavior

Some of the results obtained in muscle denervation experiments indicated that feedback from hypoglossal afferents was modulating the activity of the jaw muscles. Various techniques, including electromyography and extracellular recording from peripheral nerves were used

Fig. 2. Phylogeny of the Anura modified from Cannatella [1985]. A protrusible tongue, mandibular bending, forward lunging and a lingual withdrawal reflex are plesiomorphic characteristics of frog feeding behavior. Highly protrusible tongues, hypoglossal feedback system, tongue aiming, disjunct hyoid, hyoid protraction, hydrostatic tongue and vestigial tongue are derived characteristics.
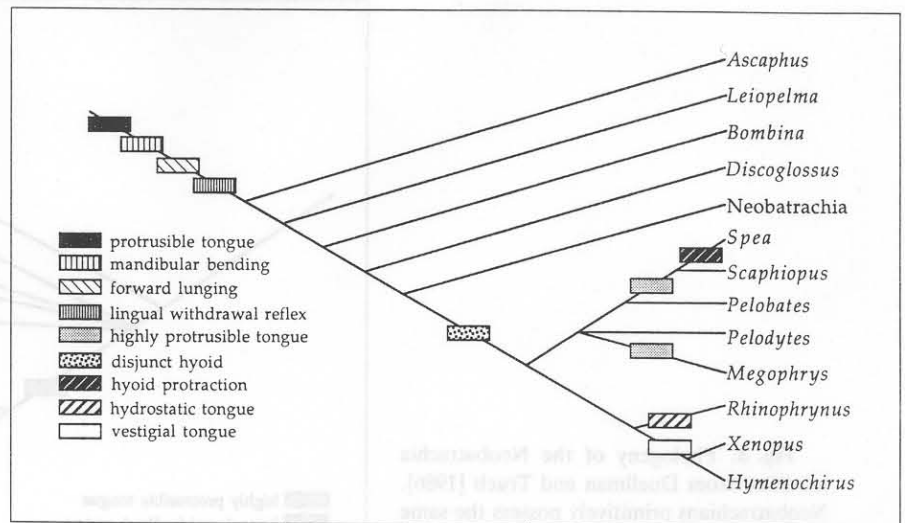
to study how hypoglossal afferents modulate feeding behavior in toads *(Bufo marinus)* and frogs *(Rana pipiens)*.

Electromyography (EMG) alone, or synchronized with video, was used to compare the activity of the mouth opening muscles (depressor mandibulae) and the mouth closing muscles (levator mandibulae) before and after surgical transection of the hypoglossal nerve in toads *(Bufo marinus)* [Nishikawa and Gans, 1990, 1992] and frogs *(Rana pipiens)* [Anderson, 1990, 1991a, b, 1992; Anderson and Nishikawa, 1992]. Electromyographic recordings of muscle activity were obtained using bipolar, Teflon™ or enamel coated, stainless steel electrodes (bared tip length = 1 mm). Electrodes were implanted percutaneously into the muscles of anesthetized subjects using 23–25 gauge hypodermic needles. External ends of the electrodes were wired into Grass HIP511E high-impedance probes, and the signals were amplified with Grass P511 H/K preamplifiers and recorded on an 8-channel tape recorder. After recovery from anesthesia, subjects were offered crickets, waxworms or earthworms. At least four feeding attempts before and after hypoglossal transection were obtained for each subject [Nishikawa and Gans, 1990, 1992; Anderson, 1990, 1991a, b, 1992; Anderson and Nishikawa, 1992].

We also recorded spontaneous activity from the ramus maxillaris of the trigeminal nerve in the levator mandibulae muscle of non-feeding, spinal-pithed toads *(Bufo marinus)*. Extracellular recordings were made from the trigeminal nerve in the body of the levator mandibulae muscle using silver, bipolar, hook electrodes. Electrodes were implanted surgically, and the incisions were closed with Nexaband™ surgical cement [Nishikawa and Gans, 1992].

Simultaneous hypoglossal transection and muscle stimulation experiments were conducted on spinal-pithed toads *(Bufo marinus)* to determine the source of the sensory feedback signal and to investigate the inhibitory effects of hypoglossal feedback on tonic activity of the jaw levators [Nishikawa and Gans, 1990, 1992]. Toads were deeply anesthetized by immersion in 10% ethanol for 30–60 min, after which the spinal cord was severed from the brain just posterior to the foramen magnum, and the spinal cord was destroyed with a dissecting needle. The geniohyoideus and sternohyoideus muscles and the hypoglossal nerve were exposed in the lower jaw. Electrical stimuli (2–5 V)

were applied with bipolar, steel electrodes at a frequency of 10 pps and a duration of 10 ms using a Grass S88 stimulator [Nishikawa and Gans, 1992].

Results

Results pertaining to the phylogenetic analysis, biomechanics, and neuromuscular control of feeding will be described in order.

Phylogenetic Analysis

Based on our phylogenetic survey of feeding kinematics (fig. 2, 3), frogs primitively possess sticky, round tongues [Nishikawa and Cannatella, 1991], which can be protruded no more than a few millimeters beyond the jaws (fig. 4a–d). Primitive characteristics of frog feeding behavior include: (1) downward bending of the lower jaw at the mentomeckelian joint during mouth opening; (2) forward lunging of the body, which brings the short tongue into contact with the prey; (3) ventroflexion of the head after prey contact; and (4) prey capture via jaw prehension [Nishikawa and Cannatella, 1991; Nishikawa and Roth, 1991; Deban and Nishikawa, 1992]. Because the tongue is short, the whole body must be launched toward the prey, and it appears from the kinematic analysis that virtually every striated muscle of the body plays a role. These ancestral characteristics are present in all of the archaeobatrachian species we have studied and also appear to be present in less

Fig. 3. Phylogeny of the Neobatrachia modified from Duellman and Trueb [1986]. Neobatrachians primitively possess the same feeding characteristics as all anurans. Highly protrusible tongues may have evolved as many as four times independently among six lineages of neobatrachians. Other derived states are the presence of a hypoglossal feedback system and tongue aiming.
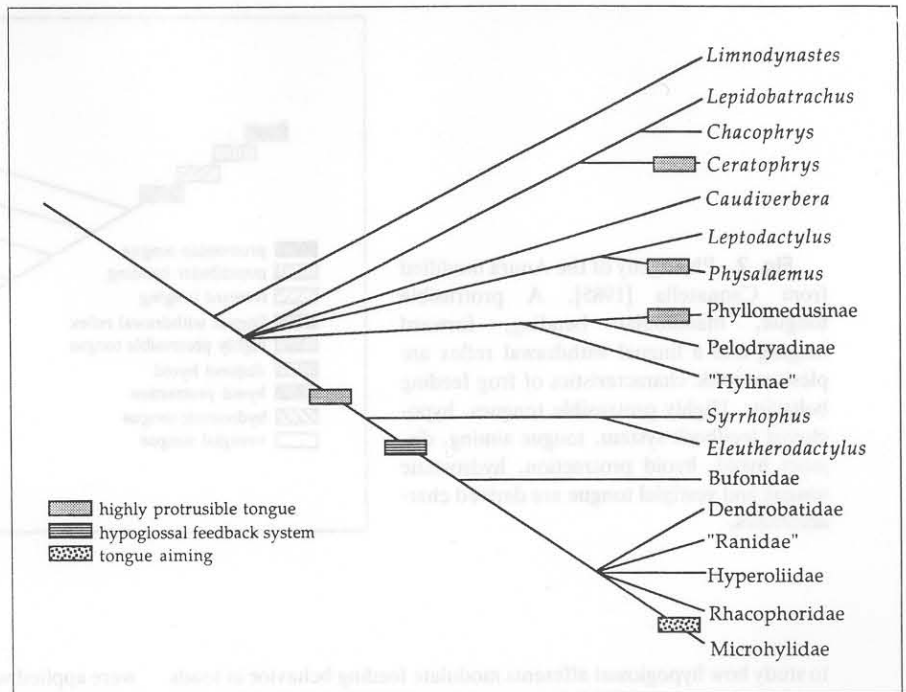
derived mesobatrachians (i.e., *Pelobates* and *Pelodytes*) [Horton, 1982; Vences, 1989] and in basal neobatrachians (i.e., *Limnodynastes*).

Highly protrusible tongues have evolved as many as six times independently from primitive tongues of limited protrusibility (fig.2, 3) [Cannatella et al., 1992]. There are seven major lineages that possess highly protrusible tongues include: (1) *Spea multiplicata* and *Scaphiopus couchii*; (2) *Megophrys montana* (fig.4e); (3) *Ceratophrys ornata*; (4) *Physalaemus pustulosus* (fig.4g); (5) *Eleutherodactylus coqui* and *Syrrhophus marnockii*, (6) the phyllomedusine hylids (fig.4f); and (7) the large lineage including the families Bufonidae, Dendrobatidae, Ranidae, Hyperoliidae (fig.4h), Rhacophoridae and Microhylidae [Cannatella et al., 1992].

In many frogs with highly protrusible tongues, there has been a tendency to reduce forward lunging during feeding behavior. The tongue, rather than the body as a whole, is projected. In these species, movements of the head and body are reduced, so that a stable platform for the tongue is formed. A parallel trend is observed in salamanders. Species with primitive tongues lunge toward prey, but the derived bolitoglossine salamanders possess highly projectile tongues and maintain a stable head and body position during prey capture [Roth, 1987; Roth et al., 1990].

Given the large number of independent events associated with the evolution of highly protrusible tongues, it is interesting to speculate on why some species have retained tongues of limited protrusibility. On the one hand, only frogs with vocal sacs have evolved highly protrusible tongues, which suggests that the tongue may be constrained from evolving in species that lack vocal sacs. On the other hand, not all frogs with vocal sacs possess a highly protrusible tongue. Conspicuous among relatively derived families is the treefrog family Hylidae, two subfamilies of which lack highly protrusible tongues. These frogs seem to have developed acrobatic lunging behavior as a prey capture strategy in lieu of a highly protrusible tongue.

Studies of feeding kinematics have been completed for four species with highly protrusible tongues: *Spea multiplicata* [Smith and Nishikawa, 1991]; *Megophrys montana* [Gans et al., 1991]; *Bufo marinus* [Nishikawa and Gans, 1992]; and *Rana pipiens* [Anderson, 1990, 1991a, b, 1992; Anderson and Nishikawa, 1992]. Each of these species exhibits special features of feeding kinematics, biomechanics and neuromuscular control that are not present in the others and thus represent evolutionary novelties.

The frog *Rana pipiens* exhibits different behavior patterns when feeding on small vs. large prey [Anderson, 1992]. When feeding on large prey, the feeding behavior of *R. pipiens* resembles that of primitive frogs [Anderson,
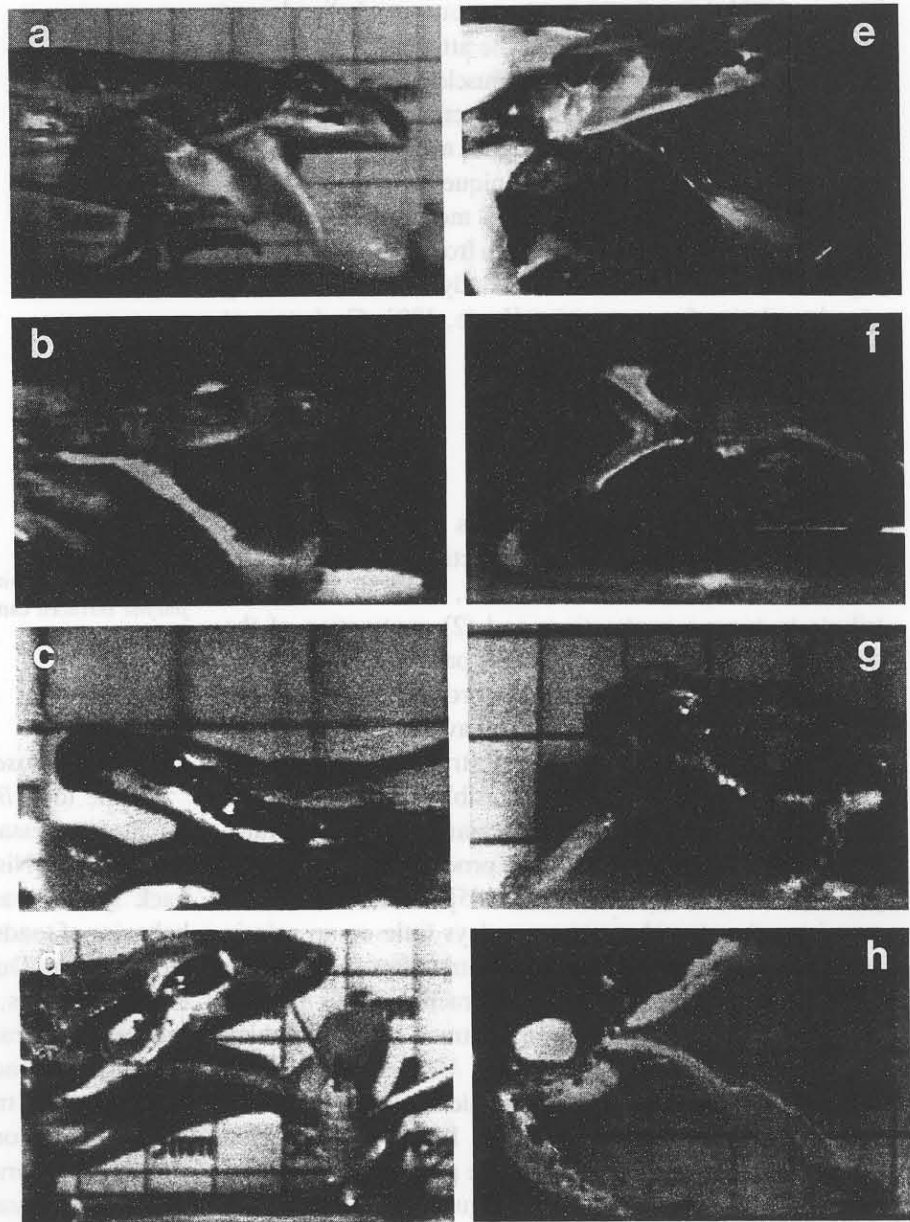
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Fig. 4. Maximum tongue protraction in frogs with protrusible tongues (**a** *Limnodynastes salmini*, a limnodynastine myobatrachid; **b** *Litoria infrafrenata*, a pelodryadine hylid; **c** *Pseudacris triseriata*, a hyline hylid; and **d** *Leptodactylus bufonius*, a leptodactyline leptodactylid) and with highly protrusible tongues (**e** *Megophrys montana*; **f** *Phyllomedusa tarsius*, a phyllomedusine hylid; **g** *Physalaemus pustulosus*, an eleutherodactyline leptodactylid; and **h** *Hyperolius* sp.).

1992]. The head is ventroflexed, the body lunges forward, the tongue is protracted through an angle of less than 90°, and the prey is captured with the jaws. When feeding on small prey, *R. pipiens* neither lunge forward nor ventroflex the head, their tongues are protracted more than 180°, and the prey is captured with the tongue only.

Lastly, we discovered that microhylids (*Phrynomerus* sp., *Dyscophus antongili*, *Kaloula pulchra* and *Gastrophryne olivacea*) can control the lateral direction of tongue protraction through an angle of at least 90° in the floor of

the mouth, independent of head and body movements (fig. 5) [Nishikawa et al., 1991]. In contrast, all other frogs we have studied orient their heads toward prey, but do not appear to regulate the direction of tongue protraction independent of head and body movements.

Microhylids possess a unique morphology of the mandible and superficial throat muscles [Emerson, 1976], which may be related to tongue aiming ability. All frogs possess a joint of varying mobility between the mentomeckelian bones and the dentary-angular elements of the mandible

[Trueb, 1973]. Microhylids possess mentomeckelian bones that have expanded surfaces for the attachment of specialized intermandibularis posterior muscles [Emerson, 1976]. We hypothesize that unilateral contraction of these muscles pivots the base of the tongue and thus aims it. Thus, microhylids appear to have evolved a unique mechanism whereby laterality of muscle contraction is modulated by analysis of prey position in the visual field. In frogs generally, orienting movements of the head and body are modulated by visual analysis of prey position [Ingle, 1983; Grobstein et al., 1985].

Biomechanics

Two biomechanical characteristics are shared by frogs from less derived lineages: (1) contraction of the submentalis muscle bends the lower downward, which does not contribute to tongue protraction; and (2) contraction of the genioglossus muscle pulls the tongue pad forward out of the mouth and is necessary for tongue protraction [Nishikawa and Roth, 1991; Deban and Nishikawa, 1992].

The biomechanics of tongue protraction differ among frog lineages with highly protrusible tongues. In the archaeobatrachian and neobatrachian frogs, the ceratohyals of the hyoid are fused to the prootic-exoccipital complex of the cranium [Cannatella, 1985]. Therefore, it is not surprising that hyoid movement plays little or no role in tongue protraction in the archaeobatrachian *Discoglossus* [unpubl. observ.] and in the neobatrachian *Bufo marinus* [Gans and Gorniak, 1982a]. In contrast, mesobatrachian frogs (including *Spea, Scaphiopus, Megophrys*) and the pipoids possess a disjunct hyoid, which is free to move in the floor of the mouth [Cannatella, 1985]. In *Spea*, as in other frogs, the genioglossus muscle pulls the tongue pad forward. However, a deficit in tongue protraction is produced when the hyoid protractor m. geniohyoideus is denervated, which indicates that hyoid protraction is necessary for tongue protraction in *Spea* [Smith and Nishikawa, 1991]. It appears that forward movement of the hyoid pushes against the tongue pad as it is pulled forward by contraction of the m. genioglossus.

Neuromuscular Control of Feeding Behavior

Neuromuscular control of feeding behavior involves hypoglossal sensory feedback, which is sometimes prey dependent, and glossopharyngeal sensory feedback.
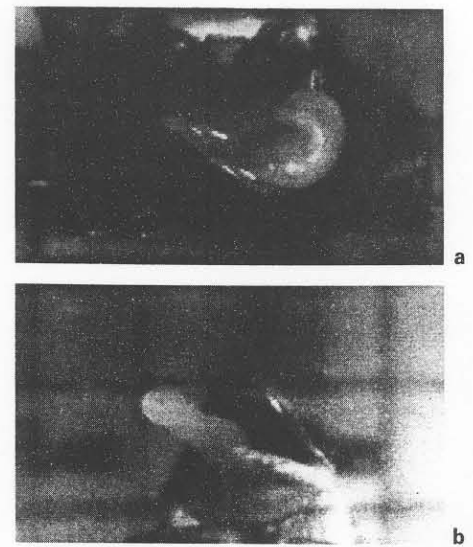
Fig. 5. The microhylids (**a**) *Phrynomerus* sp. and (**b**) *Gastrophryne olivacea* can aim their tongues relative to the lower jaw.

1. Hypoglossal Sensory Feedback

In the toad *Bufo marinus*, sensory feedback from the tongue is necessary for normal mouth opening during feeding behavior [Nishikawa and Gans, 1990, 1992]. This feedback system was discovered by comparing the feeding behavior of toads before and after transection of the hypoglossal nerve. During normal feeding, toads rotate forward on the forelimbs, open the mouth, protract the tongue onto the prey, then raise the cranium and retract the eyes, after which they retract the tongue and close the mouth (fig. 6). After bilateral transection of the hypoglossal nerve, they rotate forward on the forelimbs, retract the eyes and raise the cranium normally, but the mouth fails to open (fig. 7). Thus, hypoglossal sensory feedback must alter the activity of the mouth opening muscles (depressor mandibulae) or the mouth closing muscles (levator mandibulae), or both [Nishikawa and Gans, 1992].

During normal feeding, the mandibular depressors reach their peak activity approximately 90 ms earlier than the mandibular levators (fig. 8A). After hypoglossal transection, the mouth opening and closing muscles show synchronous peak activity (fig. 8B). Thus, it appears that the effect of hypoglossal sensory feedback is to delay the peak in mandibular levator activity by about 90 ms during normal feeding (fig. 9). This time interval corresponds to the time normally required for the mandibular depressors to open the mouth [Nishikawa and Gans, 1992].
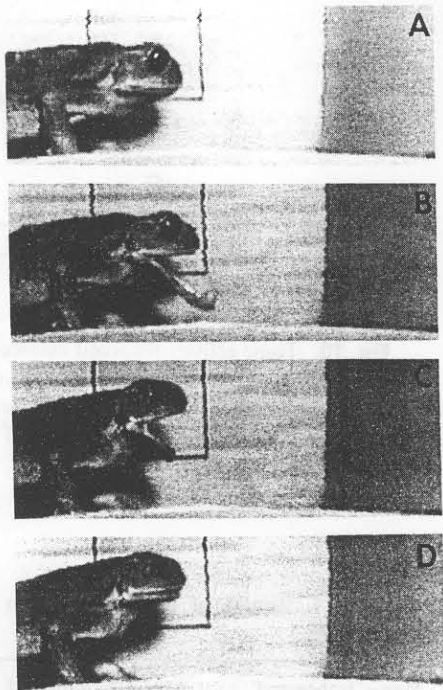
Fig. 6. Normal feeding behavior in *Bufo marinus*. These toads (**A**) rotate forward on the forelimbs, (**B**) open the mouth and protract the tongue, (**C**) retract the tongue, raise the cranium, and retract the eyes, and (**D**) close the mouth.
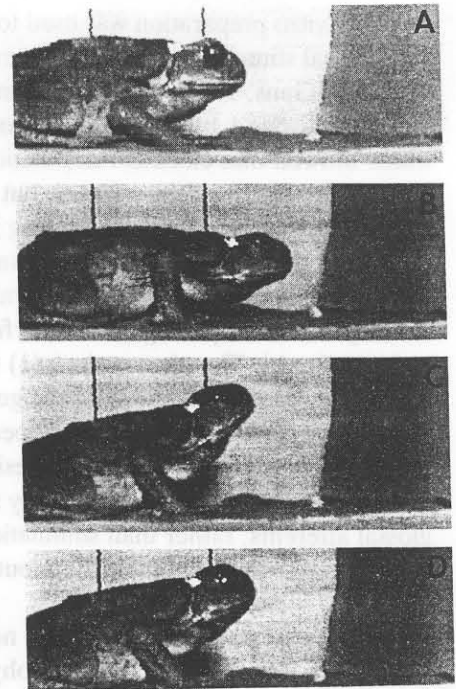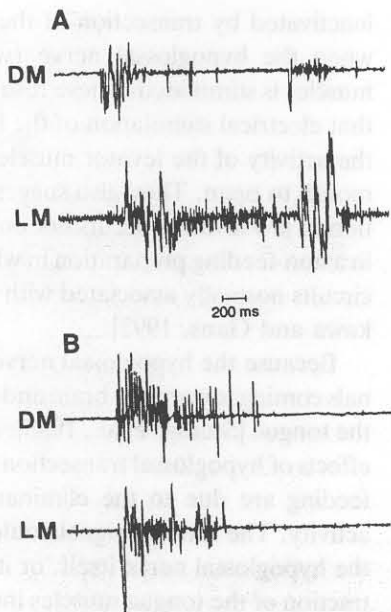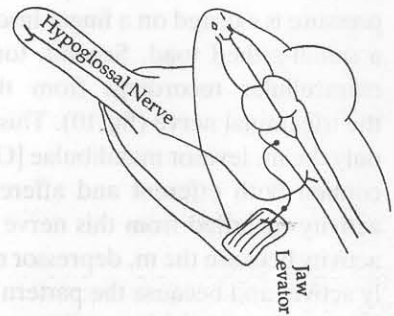

Fig. 7. Feeding behavior in *Bufo marinus* after bilateral transection of the hypoglossal nerve. These toads search actively for prey, orient toward it, and fixate it in their gaze in a normal fashion. Whenever they initiate prey capture, (**A**) they rotate forward on the forelimbs, (**B**) raise the cranium and retract the eyes normally, (**C, D**) but the mouth fails to open.

200 ms

Fig. 9. Schematic diagram of the sensory feedback system. Sensory receptors in the tongue send a signal to the brain via the hypoglossal nerve. The signal inhibits the jaw levators and thus triggers mouth opening.

Fig. 8. Electromyographic activity of the mandibular depressors (DM) and levators (LM). (**A**) Normal feeding. (**B**) Feeding after bilateral transection of the ramus hypoglossus. Before transection, the depressors reach their peak activity about 120 ms earlier than the levators. After transection, the peak activity of the levators and depressors occurs simultaneously.

An in vitro preparation was used to study the effects of hypoglossal stimulation on mouth opening in toads [Nishikawa and Gans, 1992]. The development of this preparation was inspired by an earlier report [Emerson, 1977] which showed that electrical stimulation of the geniohyoideus and sternohyoideus muscles, but not the mandibular depressors, produced mouth opening in anesthetized, spinal-pithed toads. This observation was puzzling, because the mandibular depressors are generally considered to be the major mouth-opening muscles of frogs [Gans and Gorniak, 1982a, b]. The observations (1) that the hypoglossal nerve runs through the body of the geniohyoideus muscle and (2) that hypoglossal afferents appear to inhibit mandibular levator activity led us to hypothesize that it was inhibition of mandibular levator activity by stimulation of hypoglossal afferents, rather than stimulation of the geniohyoideus muscle, that permitted the mouth to open in Emerson's [1977] experiment.

In spinal pithed toads in which no nerves have been transected, stimulation of the geniohyoideus muscle, but not the depressor mandibulae, opens the mouth (table 1), as was reported by Emerson [1977]. Stimulation of the geniohyoideus muscle after transection of the ramus hypoglossus, however, no longer results in mouth opening (table 1). Thus, stimulation of the hypoglossal nerve, not the geniohyoideus muscle (through which the nerve runs), is necessary for mouth opening [Nishikawa and Gans, 1992].

Two observations support the hypothesis that the mandibular levators are tonically active in frogs. First, positive pressure is exerted on a finger inserted between the jaws of a spinal-pithed toad. Second, tonic activity is evident in extracellular recordings from the ramus maxillaris of the trigeminal nerve (fig. 10). This nerve branch innervates only the m. levator mandibulae [Gaupp, 1896], but it could contain both efferent and afferent (spindle) axons. The activity recorded from this nerve is unlikely to be spindle activity because the m. depressor mandibulae is not tonically active, and because the pattern of activity is not random but occurs at a fairly steady rate of approximately 1 Hz (fig. 10).

Tonic activity of the mandibular levators prevents mouth opening even when the mandibular depressors are stimulated. When the mandibular levators are denervated by transection of the ramus maxillaris of the trigeminal nerve, stimulation of either the geniohyoideus or the depressor mandibulae results in mouth opening (table 1) [Nishikawa and Gans, 1992].

In these experiments, the mouth opens under only two conditions: (1) when the mandibular levators have been
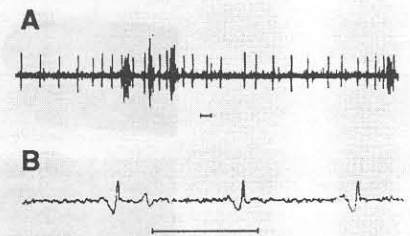

Fig. 10. Tonic activity recorded extracellularly from the trigeminal nerve in the jaw levator muscle of a spinal-pithed toad. (**A**) Low temporal resolution. The high-frequency activity is associated with mouth closing during buccal pumping. (**B**) Higher temporal resolution. Scale bars = 500 ms.

Table 1. Results of nerve transection and muscle stimulation experiments

Nerve transected	Muscle stimulated	Mouth opening?
None	depressor mandibulae	no
None	geniohyoideus	yes
Ramus hypoglossus	depressor mandibulae	no
Ramus hypoglossus	geniohyoideus	no
Ramus maxillaris	depressor mandibulae	yes
Ramus maxillaris	geniohyoideus	yes

inactivated by transection of the ramus maxillaris; or (2) when the hypoglossal nerve (within the geniohyoideus muscle) is stimulated. These results support the hypothesis that electrical stimulation of the hypoglossal nerve inhibits the activity of the levator muscles, thereby permitting the mouth to open. They also suggest that hypoglossal inhibition of jaw levators occurs not only during feeding but also in a non-feeding preparation in which none of the premotor circuits normally associated with feeding are active [Nishikawa and Gans, 1992].

Because the hypoglossal nerve carries both sensory signals coming toward the brain and motor signals going out to the tongue [Stuesse et al., 1983], it is uncertain whether the effects of hypoglossal transection on mouth opening during feeding are due to the elimination of sensory or motor activity. The sensory signal could enter the brain through the hypoglossal nerve itself, or it could be a result of contraction of the tongue muscles innervated by the hypoglossal nerve and enter the brain through another cranial

nerve. Only two nerves ramify in the region of the genio-glossus medialis and genioglossus basalis muscles: (1) the ramus palatinus of the trigeminal and facial nerves innervates the maxilla, against which the tongue may be pushed when these muscles contract; and (2) the ramus lingualis of the glossopharyngeal nerve ramifies within the tongue. The ramus palatinus and the ramus lingualis were also transected in spinal-pithed toads to determine if either ramus carries sensory signals that inhibit the activity of the m. levator mandibulae. However, transection of neither ramus abolished mouth opening when the geniohyoideus muscle was stimulated. Thus, only the hypoglossal nerve carries sensory input that inhibits the m. levator mandibulae [Nishikawa and Gans, 1992]. Anatomical studies in the frog *Rana pipiens* have demonstrated that the hypoglossal nerve is composed of both sensory and motor fibers [Stuesse et al., 1983]. However, the central projections and the sensory receptors associated with the hypoglossal sensory feedback system remain to be described.

It seems clear that the hypoglossal sensory feedback system of *Bufo marinus* is a mechanism for coordinating tongue protraction and mouth opening. There are at least three non-exclusive hypotheses for the functional advantage of such a peripheral tongue-jaw coordination mechanism. (1) The tongue is protracted before the mouth opens, and mouth opening releases the 'pre-loaded' tongue (the 'jack in the box' hypothesis). (2) The tongue is attached to the lower jaw, which moves through a downward trajectory during feeding. Aiming of the tongue may require precise coordination of tongue protraction with lower jaw movement. (3) In toads and many other anurans, the tongue can be protracted to a distance that is greater than the gape. Tongue-jaw coordination may function to insure that, as the tongue rises from the floor of the mouth during protraction, it does not hit the upper jaw.

Archaeobatrachian and neobatrachian frogs that lack highly protrusible tongues, including *Discoglossus pictus* [Nishikawa and Roth, 1991], *Bombina orientalis* [unpubl. observ.] and *Hyla cinerea* [Deban and Nishikawa, 1992] appear to lack the hypoglossal feedback system, as does *Spea multiplicata* [Smith and Nishikawa, 1991], although it has a highly protrusible tongue. Among neobatrachians, only *Rana pipiens* and *Bufo marinus* have so far been found to possess the feedback system [Nishikawa and Gans, 1992; Anderson, 1991b]. The presence of hypoglossal feedback in derived frog species and its absence in primitive species indicate that it is a derived feature within frogs.

2. Prey-Type Dependent Hypoglossal Sensory Feedback

Unlike *Bufo marinus*, which has the longest tongue relative to jaw length of any known frog [Cannatella et al., 1992] and prefers small prey [Duellman and Trueb, 1986], the leopard frog *Rana pipiens* is somewhat more of a dietary generalist and modulates its feeding behavior in response to prey type [Anderson, 1992]. The ability to modulate feeding behavior in response to prey type is widespread among frogs with highly protrusible tongues [unpubl. observ.], and this ability appears to be reduced or absent in *Bufo*, perhaps because of its preference for small prey.

When feeding on small prey such as waxworms, *Rana* uses tongue protraction to catch prey, and minimizes head and body movements. When feeding on large prey such as earthworms, it lunges forward with its body, flexes the head downward, exhibits reduced tongue protraction and uses jaw prehension to catch prey [Anderson, 1992]. Preliminary studies show that *Rana* responds to small pieces of earthworm as though they were waxworms. Thus, it appears that visual analysis of prey size or shape leads to the expression of the different motor patterns used for different prey types [Anderson, 1992].

The mechanism that coordinates mouth opening and tongue protraction also varies with prey size in *Rana* [Anderson, 1991b]. When feeding on small prey, sensory feedback from the tongue through the hypoglossal nerve is necessary to trigger mouth opening, as in *Bufo marinus*. If sensory feedback is prevented from reaching the brain by transection of the hypoglossal nerve, then the mouth fails to open, and the mouth opening and closing muscles reach their peak activity simultaneously, as in *Bufo* (fig. 7, 8). In contrast, when feeding on large prey, the mouth opens normally after hypoglossal transection, and the jaw levators and depressors do not reach their peak activity simultaneously [Anderson, 1991b; Anderson and Nishikawa, 1992]. This indicates that hypoglossal sensory feedback is not necessary to trigger mouth opening in *Rana* when feeding on large prey. This latter result is also found in primitive frog species [Nishikawa and Roth, 1991].

3. Glossopharyngeal Sensory Feedback

In the frogs *Ascaphus truei* [Nishikawa and Cannatella, 1991], *Discoglossus pictus* [Nishikawa and Roth, 1991], *Hyla cinerea* [Deban and Nishikawa, 1992] and *Bombina orientalis* [unpubl. observ.], tongue retraction begins 16–40 ms after prey contact. When the prey is not contacted, these frogs protract their tongues a second or third time before closing the mouth. Tongue retraction and mouth closing may be delayed by 40–100 ms. These observations

are consistent with the hypothesis that sensory feedback associated with prey contact triggers tongue withdrawal and mouth closing in these species.

Matsushima et al. [1986, 1988] found that stimulation of the ramus lingualis of the glossopharyngeal nerve in *Bufo japonicus* produced excitatory postsynaptic potentials in both ipsilateral and contralateral tongue retractor motor neurons, but only connections with the ipsilateral tongue retractor motor neurons were monosynaptic [Matsushima et al., 1987, 1988]. Reticular interneurons also receive glossopharyngeal input [Matsushima et al., 1989]. The latency of the monosynaptic pathway was 12 ms in *Bufo*, including conduction time from the tongue to the brain and back to the tongue [Matsushima et al., 1987, 1988]. Given 4–5 ms for excitation/contraction coupling in the muscle, this corresponds nicely with the 16 ms interval between prey contact and onset of tongue retraction observed kinematically [Nishikawa and Gans, 1992]. Kumai [1981] reported that electrical and chemical stimulation of the tongue evokes discharges in the hypoglossal nerve, which innervates the tongue protractors and retractors, although the relationship of these discharges to normal feeding behavior remains unclear. These studies demonstrate that the tongue retractor motor neurons of toads receive excitatory input from glossopharyngeal sensory neurons through both monosynaptic and polysynaptic pathways.

The presence of a tongue withdrawal reflex in a wide variety of frog species suggests that this feedback system is a primitive feature of frogs (fig. 2). However, it seems to be absent in some frogs (e.g., *Megophrys montana*) and in salamanders. In *Megophrys montana*, the onset of tongue retraction may not occur until 300 ms after prey contact [Gans et al., 1991], and several studies of salamanders have failed to find differences in onset of tongue withdrawal between successful and unsuccessful capture attempts [Larsen et al., 1989; Findeis and Bemis, 1990; Reilly and Lauder, 1990; Roth et al., 1990]. Furthermore, in *Bolitoglossa subpalmata* [Thexton et al., 1977], the tongue retractor muscles become active before prey contact.

Discussion

The recent kinematic studies reviewed here have shown that highly protrusible tongues have evolved numerous times independently from ancestral tongues of limited protrusibility [Cannatella et al., 1992]. Some lineages of frogs with highly protrusible tongues have evolved unique biomechanical mechanisms for protracting their tongues [Smith and Nishikawa, 1991], and/or unique neural mechanisms for coordinating feeding behavior [Nishikawa and Gans, 1992; Anderson, 1991b; Anderson and Nishikawa, 1992]. The biomechanics and neuromuscular control of feeding behavior have been transformed repeatedly during frog evolution.

Much work remains to be done to elucidate novel mechanisms of neuromuscular control in many frog lineages. Electromyography and nerve transection experiments have been performed for only a few species. From the few studies that have been done, it is clear that the present categorization of tongue types in frogs (i.e., discoglossoid, aglossal, hydrostatic and anteriorly attached) is not sufficient to describe the diversity of feeding behavior that frogs exhibit. However, we can begin to draw generalizations about the components of the motor system that have evolved in the species that have been studied. Because the patterns of muscle activity during feeding behavior have only been studied in the genus *Bufo*, it is not yet possible to say whether or not muscle activity patterns have evolved conservatively in this system. However, our studies do suggest that there has been much evolution at the interface between central pattern generators and sensory inputs in the motor system for feeding in frogs.

In primitive frogs, hypoglossal sensory input is not necessary for mouth opening during feeding [Nishikawa and Roth, 1991; Deban and Nishikawa, 1992]. In the absence of hypoglossal feedback, the motor pattern is apparently one in which the mouth opening muscles are active before the mouth closing muscles. The primitive behavior pattern is also observed in *Spea multiplicata*, a mesobatrachian with a highly protrusible tongue [Smith and Nishikawa, 1991]. However, in the neobatrachians *Bufo marinus* [Nishikawa and Gans, 1992] and *Rana pipiens* [Anderson, 1991b; Anderson and Nishikawa, 1992], hypoglossal input is necessary for mouth opening during feeding. When hypoglossal input is prevented from reaching the brain, the motor pattern is one in which the mouth opening and closing muscles are active simultaneously. Thus, it appears that hypoglossal input normally inhibits the activity of the mouth closing muscles during mouth opening in these species.

The behavior patterns used by *Rana pipiens* to catch large and small prey correspond to differences in feeding behavior between primitive and derived frogs, respectively. Primitive frogs have short tongues that can be protracted only a few millimeters (fig. 4a–d), so these frogs must lunge toward prey to catch them. In contrast, derived frogs have highly protrusible tongues and do not need to lunge forward to catch prey (fig. 4e–h). The similarity between movements used by *Rana* to catch large prey and

those used by primitive frogs in general suggests that *Rana* has retained the plesiomorphic motor pattern and uses it to catch large prey, while acquiring a derived motor pattern to catch small prey [Anderson, 1992].

Furthermore, hypoglossal feedback is only necessary for mouth opening in *Rana* when feeding on small prey [Anderson, 1991b; Anderson and Nishikawa, 1992]. This result implies that the role of hypoglossal sensory input during feeding is modified by incoming sensory input associated with visual assessment of prey size [Anderson, 1991b]. We hypothesize that tectal output differs with prey size in *Rana*, and that different populations of premotor reticular neurons (fig. 1) are activated for prey of different sizes. One hypothesis that would explain why hypoglossal feedback is necessary for mouth opening only when the frogs feed on small prey is that only those premotor reticular neurons activated by 'small prey tectal output' receive input from hypoglossal afferents.

The hypoglossal sensory feedback system is present in both *Bufo marinus* and *Rana pipiens*. However, we have been unable to observe prey-type dependence of hypoglossal feedback in *Bufo marinus*, although we have fed the same types of prey to both species. Most frogs are generalist predators, feeding on a wide range of prey items [Toft, 1981], and the ability to modulate feeding behavior in response to prey type appears to be widespread among frogs with highly protrusible tongues [unpubl. observ.]. It appears that the ability to modulate feeding behavior in response to prey type has been reduced or lost in *Bufo*, perhaps because of the preference of bufonids for relatively small prey [Emerson, 1985].

From these experiments, it appears that there has been considerable evolution at the interface between reticular central pattern generators associated with feeding and sensory feedback circuits that modulate feeding motor output. Among frogs, the roles of hypoglossal and glossopharyngeal sensory feedback appear to be relatively plastic in their evolution. Furthermore, prey-type dependence of hypoglossal sensory feedback suggests that the interaction between descending visual control and sensory feedback may also be evolutionarily plastic.

In contrast, the feeding behavior of salamanders appears to have evolved much more conservatively than that of frogs, despite great morphological diversity of the feeding apparatus among salamanders [Roth et al., 1990]. Lombard and Wake [1976, 1977] described eight modes of tongue morphology among salamanders of the family Plethodontidae. The modes differ in the degree to which the tongue is attached to the anterior margin of the mandible and in the degree of tongue protraction. Although salamanders differ greatly in tongue morphology, the available evidence suggests that motor patterns are highly conserved among salamanders. Electromyographic data are available for only two species of salamanders: *Bolitoglossa occidentalis*, a species with a highly derived, projectile tongue [Thexton et al., 1977] and *Ambystoma tigrinum*, a species with a primitive, anteriorly attached tongue [Lauder and Shaffer, 1985]. Although tongue morphology is highly divergent in these species, the pattern of motor activity during feeding is the same. In both salamanders, all muscles involved in feeding are activated simultaneously [Thexton et al., 1977; Lauder and Shaffer, 1985], whereas among frogs the pattern of muscle activation is much more complex [Gans and Gorniak, 1982a, b; Matsushima et al., 1985].

There is also evidence from amphibians that the interface between the CPGs of the reticular formation and descending control from the optic tectum may be subject to evolutionary change. In anurans, stimulation of the crossed tectobulbospinal tracts evokes feeding behavior [Ewert, 1967]. The pattern of motor activity in jaw and tongue muscles that is evoked by tectal stimulation is similar to that observed during normal feeding [Matsushima et al., 1985]. Tectobulbar and tectospinal fibers are presynaptic to reticular interneurons, which are presynaptic to feeding motor neurons. In frogs, all connections between tectobulbar and tectospinal fibers and feeding motor neurons are polysynaptic, and most are inhibitory [Matsushima et al., 1989]. In contrast, stimulation of tectobulbar and tectospinal tracts in salamanders resulted in stable responses of short latency (2–3 ms), and the responses also followed repetitive shocks at short latency, indicating that the connection between descending tectal input and motor neurons is probably monosynaptic [G. Roth and T. Matsushima, personal communication]. Thus, descending control of feeding behavior appears to differ considerably between frogs and salamanders [Roth et al., 1990]. The direct connection between the optic tectum and feeding motor neurons may be responsible for the pattern of simultaneous muscle activity observed in salamanders, as well as for differences in motor control of feeding behavior between salamanders and frogs.

The reticular formation is a major premotor integration area of vertebrates that has been implicated in the generation of motor patterns for a number of behaviors. The gigantocellular nucleus of the reticular formation is the major source of reticulospinal fibers that modulate the activity of brainstem and spinal motor neurons, and it may thus contribute to the generation of patterned motoneuronal activity. The gigantocellular nucleus has been shown to contain interneurons involved in the generation of masticatory rhythms in mammals [Nozaki et al., 1986; Lund and

Enomoto, 1988] and of feeding motor patterns in frogs [Matsushima et al., 1989]. It may be involved in the generation of vertebrate locomotor rhythms as well [J. Fetcho, personal communication].

In many ways, the reticular formation appears to fit the definition of a highly integrated, multifunctional structure. The reticular formation takes its name from the inordinately widespread connections established by its individual interneurons [Kandel and Schwartz, 1981], which may send axon collaterals to all major subdivisions of the brain, with extensive ramifications in the brainstem. This pattern of connections is far more complex than that of the descending tectobulbospinal tracts, and it is more complex than most, if not all, primary sensory projections. The widespread connections of the reticular formation imply a high level of integration, as well as involvement in multiple functions.

We hypothesize that the gigantocellular nucleus of the reticular formation may evolve conservatively, because it is a highly integrated, multi-functional network. If individual CPG interneurons are widely connected to multiple targets, and are involved in the generation of rhythmic activity associated with many different behavior patterns, then one would expect that these neurons would evolve conservatively, because they could not respond to selection for optimizing one motor pattern without compromising other functions.

One caveat that cannot be overemphasized is that CPGs have evaded anatomical description in vertebrates for the past 25 years, although recent progress has been made in elucidating intraspinal CPG circuits as evidenced in this workshop [Fetcho, 1992]. Despite this problem, there does seem to be some empirical evidence for evolutionary conservatism of the reticular formation. In a comparative study of vertebrate classes, the gigantocellular nucleus was found to be the most highly conserved nucleus of the reticular formation [Cruce and Newman, 1984]. Recent studies also suggest that intraspinal locomotor CPG circuits have been conserved among anamniotes [Fetcho, 1992].

A second caveat is that the gigantocellular nucleus of the reticular formation may not be representative of CPGs, in general, or even of vertebrate CPGs, in particular. Until we know more about the anatomical organization of vertebrate CPG networks, it will be impossible to make generalizations about their evolution. Furthermore, CPG networks underlying feeding behavior may differ from those controlling continuous movements, such as locomotion or respiration. In particular, one might expect that CPGs for intermittent or rare behavior patterns are more subject to modulation by incoming sensory input than are continuous behavior patterns. Thus, our results on the evolution of the frog feeding motor system may not generalize to other types of behavior patterns.

In summary, comparative studies have found that motor systems may evolve conservatively across morphological and behavioral transitions [Lauder and Shaffer, 1988; Goslow et al., 1989] or may be subject to considerably more evolutionary change than morphological characteristics [Liem, 1978; Lauder, 1983; Gordon and Herring, 1987]. In the case of frog feeding behavior, these observations can be explained by the hypothesis that the gigantocellular nucleus of the reticular formation may evolve conservatively, because it is a highly integrated, multifunctional network that probably cannot be optimized for one function without compromising others. In contrast, the interfaces between the reticular formation, sensory feedback and descending control may be less constrained. This hypothesis predicts that when an evolutionary change in feeding motor patterns has occurred during evolution [e.g., Lauder, 1983; Gordon and Herring, 1987], it is likely that sensory feedback or descending control may be involved.

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Note added in proof

Weerasuriya [in Visuomotor Coordination: Amphibians, Comparisons, Models and Robots, ed. by J.-P. Ewert and M.A. Arbib, 1989, pp. 589–614] noted that the mouth also fails to open after bilateral hypoglossal transection in the toad Bufo bufo.