

MECHANISMS OF TONGUE PROTRACTION AND NARIAL CLOSURE IN THE MARINE TOAD *BUFO MARINUS*

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Summary

Electromyography, kinematic analysis, muscle stimulation and denervation techniques were used to investigate the muscular mechanisms of narial closure during breathing and of tongue protraction during prey capture in the marine toad *Bufo marinus*. Toads were video-taped during breathing and feeding under a variety of conditions: before surgery, after unilateral or bilateral denervation of the *M. submentalis*, and after unilateral or bilateral denervation of the *Mm. genioglossus basalis* and *medialis*. Deeply anesthetized toads were video-taped during stimulation of several cranial muscles, and electromyograms were recorded from the *M. submentalis* during feeding before and after its denervation. *Bufo marinus* differs from many other anurans in having a relatively long tongue that experiences large accelerations (>31 g) during protraction. Tongue protraction occurs in two phases: an early phase during which the lingual tip moves upward and forward relative to the mandibular tip as the tongue shortens, and a later phase during which the

lingual tip moves downward and forward relative to the mandibular tip as the tongue elongates under its own momentum. Relative to an external reference, the lingual tip follows a straight trajectory from mouth to prey, which depends critically upon precise coordination of tongue and jaw movements. The *M. submentalis* is necessary for narial closure during breathing, but is unnecessary for normal tongue protraction during feeding. In contrast, the *Mm. genioglossus basalis* and *medialis* are necessary for forward movement of the tongue pad over the symphysis. In *B. marinus*, a simple anatomical change (elongation of the tongue) has functional consequences (inertial elongation) that profoundly affect the mechanisms of neuromuscular control. Though seldom studied, it seems likely that morphological evolution has had a profound influence on mechanisms of motor control in animals generally.

Key words: denervation, kinematics, feeding behavior, tongue protraction, breathing, frogs, *Bufo marinus*.

Introduction

A major goal of functional morphology is to understand the biomechanical implications of anatomical changes during evolution (Gans, 1980; Hildebrand *et al.* 1985). An example is Webb's (1984) study of the effects of differences in anatomy between eels and tuna on the kinematics of swimming. Less often addressed are the changes in mechanisms of motor control in morphologically divergent organisms. In recent studies, we have pursued this question using the prey-capture behavior of anurans as a model system. These studies have included anatomical (Regal and Gans, 1976), behavioral (Anderson, 1993), biomechanical (Gans and Gorniak, 1982*a,b*), neurological (Nishikawa and Gans, 1992; Anderson and Nishikawa, 1993) and evolutionary (Nishikawa *et al.* 1992) studies of the anuran feeding apparatus. The purpose of the present study is to use a multidisciplinary approach to refine our current understanding of the biomechanics and neural control

of feeding and breathing in the marine toad *Bufo marinus* and to explore the transitions involved in their evolution.

The tongues of most anurans are attached anteriorly near the mandibular symphysis. The posterior margin of the tongue lies freely in the floor of the mouth. During feeding, the base of the tongue is rotated over the symphysis, so that the dorsal surface of the tongue at rest becomes the ventral surface at full protraction. It is this surface that makes contact with the prey (Magimel-Pelonnier, 1924; Regal and Gans, 1976; Horton, 1982). In anurans, the placement of the tongue in the anterior part of the oral cavity has been hypothesized to reduce functional conflicts that might arise between feeding and pulse pumping ventilation in the buccal cavity (Gans, 1990).

The initial studies that led to an explanation of the muscular basis of the mechanism of tongue projection in anurans were derived from kinematic analyses and muscle stimulation

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experiments in *Rana catesbeiana* (Gans, 1952; Severtzov, 1961). Later, additional species were studied (Gans, 1961; Regal and Gans, 1976). A ballista model of tongue protraction, developed originally for the American bullfrog *Rana catesbeiana* (Regal and Gans, 1976), was later tested using electromyography in the marine toad *Bufo marinus* (Gans and Gorniak, 1982*a,b*). This model (Gans and Gorniak, 1982*a,b*) suggests that the *M. genioglossus medialis* forms a stiffened rod and that the *M. genioglossus basalis* forms a wedge at the anterior end of the rod near the mandibular symphysis. Activation of the *M. submentalis* lifts the middle of the lingual rod over and about the mandibular symphysis and also depresses the symphysis (Gans and Gorniak, 1982*a,b*).

On the basis of the ballista hypothesis, it appears that feeding involves some of the same structures posited to close the nares during ventilation, specifically the *M. submentalis* (Gaupp, 1896*a*; de Jongh and Gans, 1969). Gaupp (1896*a*) hypothesized that this muscle closes the nares indirectly by lifting the mentomeckelian bones, which themselves deform the alary cartilages and effect narial closure.

The goal of the present study is to extend our previous work on the feeding and ventilatory mechanisms of the marine toad. Using electromyography combined with nerve transection, a recent analysis of the neural control of feeding in *Bufo marinus* disclosed that the coordination of tongue and jaw movements during feeding is more complex than previously thought because opening of the mouth requires sensory feedback from the tongue (Nishikawa and Gans, 1990, 1992). Here, we use a combination of techniques (including kinematic analysis, electromyography, muscle stimulation and denervation) to further our understanding of the contribution of the cephalic muscles to feeding and ventilation in the marine toad *Bufo marinus*. These studies form the basis for exploring the evolution of the toad tongue compared with that of more basal anurans and provide significant new insights into the relationship between morphological change and neuromuscular control of the anuran feeding apparatus.

Materials and methods

Fifteen adult *Bufo marinus* L. (94–401 g), obtained from

animal suppliers, were used in this study. Two toads were used for electromyography, five were used for muscle stimulation and eight were used for kinematic analysis and denervation experiments. All toads received only one treatment, except toad 2 which received first unilateral and then bilateral denervation of the *M. submentalis*. The masses and snout–vent lengths of the eight toads included in the kinematic analysis are given in Table 1. Several procedures were used to investigate the mechanisms of tongue protraction during prey capture and narial closure during breathing. Electromyography was used to study the activity of the *M. submentalis* during feeding, both before and after denervation of this muscle. Electrical stimulation was used to investigate the function of several cranial muscles, including the *M. submentalis* and *Mm. genioglossus medialis* and *basalis*. In addition, toads were video-taped during feeding and breathing before and after unilateral or bilateral denervation of the *M. submentalis* or *Mm. genioglossus medialis* and *basalis*. For the feeding sequences, computer motion-analysis was used to analyze the effects of denervation. For the breathing sequences, the movements of the tiny nares were scored from video tapes as: (1) narial movement with complete narial closure; (2) narial movement but with incomplete closure; or (3) no narial movement and no closure. We use upper case *N* to indicate the sample size of individual toads and lower case *n* to indicate the sample size of feeding or breathing sequences video-taped for each individual. Voucher specimens of *Bufo marinus* have been deposited in the Museum of Zoology of the University of Michigan (UMFS no. 7719-20).

Electromyography and muscle stimulation

The muscles of the feeding apparatus in *Bufo marinus* are shown in Fig. 1. For electromyography, patch electrodes (Loeb and Gans, 1986) were sutured onto the ventral surface of the *M. submentalis* in two toads. The electrodes were constructed by sewing two stainless-steel wires (0.075 mm diameter) into a 4 mm×4 mm×0.5 mm Silastic sheet. The wires were placed to form a 1 mm bipole with 1 mm of uninsulated recording surface on each wire. Electromyograms (EMGs) were recorded during feeding before (*n*=9 sequences for one toad and *n*=3 sequences for the other) and after (*n*=10 sequences for one toad)

Table 1. Numbers of feeding sequences analyzed for *Bufo marinus* for each of the surgical denervation treatments

Individual	Body mass (g)	SVL (mm)	Before surgery	Unilateral submentalis	Bilateral submentalis	Unilateral genioglossus	Bilateral genioglossus
1	163	108	3C	–	–	–	–
2	140	112	–	3C	3C	–	–
3	94	100	3C	–	1C	–	–
4	146	105	3C	–	3C	–	–
5	303	132	1C, 1U	–	–	3U	–
6	144	104	3C	–	–	–	3U
7	362	139	3C	–	–	–	3U
8	338	136	3C	–	–	–	3U

SVL, snout–vent length; C, successful captures; U, unsuccessful capture attempts.

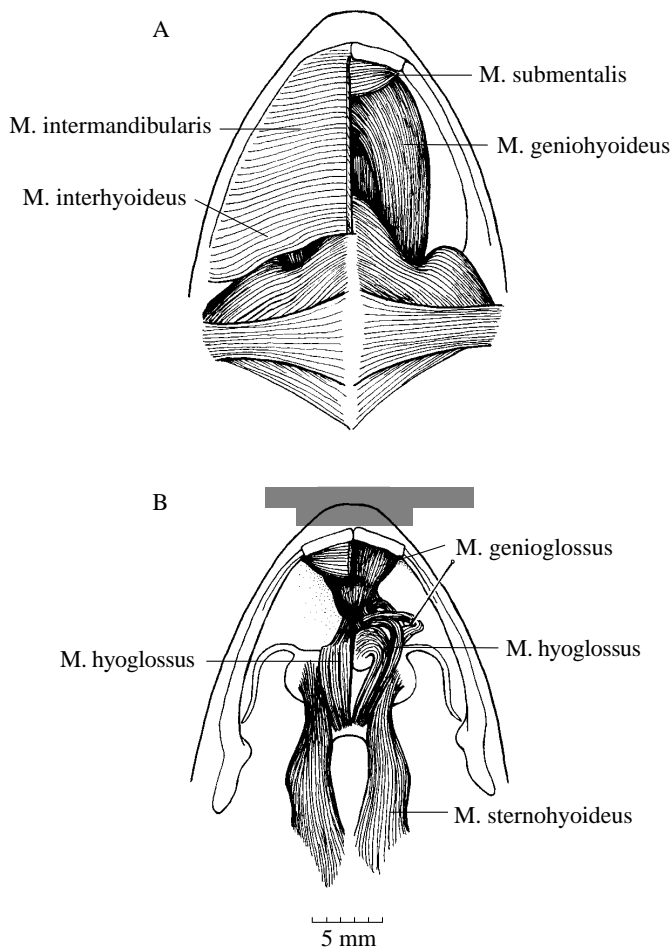


Fig. 1. Muscles of the feeding apparatus in *Bufo marinus*. (A) Superficial muscles of the feeding apparatus include M. intermandibularis, M. interhyoideus, M. submentalis and M. geniohyoideus. (B) Deep muscles of the feeding apparatus include M. sternohyoideus and the two extrinsic tongue muscles Mm. hyoglossus and genioglossus.

denervation of the M. submentalis. The signals were amplified 1000-fold and recorded on FM tape using a Honeywell 101 tape recorder at a speed of 19 cm s^{-1} (bandpass $>100 \text{ Hz}$ and $<2500 \text{ Hz}$).

For muscle stimulation, two toads were deeply anesthetized and selected cranial muscles were stimulated, either alone or in various combinations, using a capacitance-coupled stimulating electrode (1 mm bipole) while the preparation was video-taped. The stimulated muscles included the M. submentalis, M. genioglossus medialis, M. hyoglossus, M. geniohyoideus, M. depressor mandibulae and M. adductor mandibulae. The stimulation regime was monophasic at 50 Hz, 10 ms pulse duration, 10 ms interpulse interval and 1–6 V. The head was held with the mouth open, the mandible in a horizontal position and the cranial axis positioned at 30° above the horizon. Because the muscles fatigued extremely rapidly, we used minimal stimulating voltage (1 V) and attempted to video-tape the observations of the first stimulation trial.

The M. submentalis was stimulated in three ways: (1) directly with a 1.5 mm double-wire, bare electrode placed on the surgically exposed ventral surface of the muscle; (2) with a pair of 1 cm twin-rod electrodes placed either on the ventral integument or lateral to the mucous membrane of the buccal cavity; or (3) *via* the ramus mandibularis of the N. trigeminus. In the latter case, the N. trigeminus was freed surgically, isolated electrically by placing a Silastic sheet between the nerve and the underlying tissues, and then holding the nerve with the pair of electrodes. The M. genioglossus medialis was stimulated *via* electrodes applied to the external surface of the tongue through the open mouth and *via* electrodes inserted into the belly of the muscle. One electrode was placed near the free distal portion of the muscle and the other was placed near the symphysis.

Muscle denervation

To determine the sites for denervation, we cleared several specimens of *B. marinus* and stained the peripheral nerves with Sudan Black B (Nishikawa, 1987). The M. submentalis is innervated by the ramus mandibularis of the trigeminal nerve (Fig. 2A), which carries both sensory and motor fibers (Gaupp, 1896b). The Mm. genioglossus basalis and medialis are innervated by the most distal branches of the ramus hypoglossus of the second spinal nerve (Fig. 2A), which also carries sensory and motor fibers (Nishikawa and Gans, 1992; Stuesse *et al.* 1983). For denervation, the toads were anesthetized by immersion in 10–30% ethanol or 0.1% MS222 (tricaine methanesulfonate, 1 g l^{-1} tap water) for 10–60 min. The ramus mandibularis was exposed along the dentary and transected distal to the branch that innervates the M. intermandibularis (Fig. 2A, open arrow). A 2 mm length of the nerve was excised and the incision was closed with Nexaband surgical adhesive.

Denervation of the Mm. genioglossus basalis and medialis proceeded similarly. The ramus hypoglossus was transected surgically in the mandibular region, distal to the branch that innervates the M. geniohyoideus (Fig. 2A, filled arrow). After the hypoglossal nerves have been transected bilaterally in *Bufo marinus*, the toads no longer open their mouths during feeding because sensory feedback has been interrupted (Nishikawa and Gans, 1992). Thus, the function of the Mm. genioglossus basalis and medialis could not be tested initially. However, it was later discovered that toads with hypoglossal transection eventually learn to open their mouths and attempt to feed and at this stage the roles of the Mm. genioglossus basalis and medialis during feeding were tested.

Motor learning following hypoglossal transection is the topic of ongoing studies (Innocenti and Nishikawa, 1994). However, some of the details are relevant for the present analysis. These studies demonstrate that there is an initial period following hypoglossal transection during which the toads attempt to feed but before they begin to modify their behavior. No measurable learning occurs during the first 10–12 feeding attempts after hypoglossal transection. Furthermore, learning to open the mouth appears to have only a small effect

on tongue movements. In the first few trials after hypoglossal transection, tongue protraction is reduced to approximately 10% of the distance before transection. Tongue protraction distance increases somewhat with practice, up to as much as 33% of normal. When the Mm. genioglossus basalis and medialis are reinnervated by the regenerating hypoglossal nerves, tongue protraction distance increases rapidly. This occurs approximately 30 days after transection. Because tongue protraction distance improves to some degree with practice, the effects of motor learning will tend to make it more, rather than less, difficult to demonstrate that denervation of the the Mm. genioglossus basalis and medialis has an effect on tongue movements.

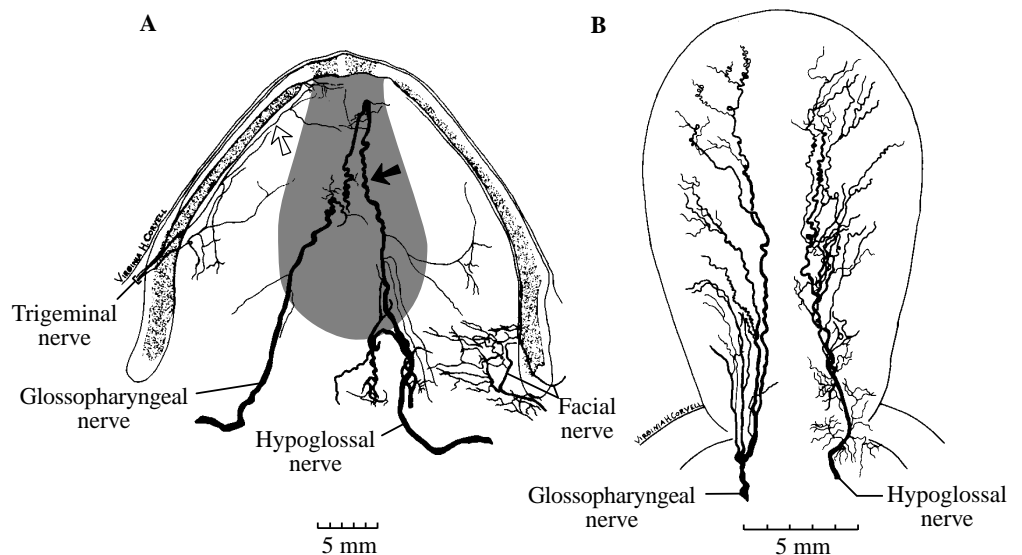
Three post-surgical feeding attempts in which the mouth was opened were digitized and analyzed for each toad ($N=7$). Of the three toads with hypoglossal transection included in the present study, one (toad 6, Table 1) learned to open its mouth on the sixteenth trial over 5 days, the second (toad 7, Table 1) learned to open its mouth after more than 35 trials over 25 days, and the third (toad 8, Table 1) learned to open its mouth on the thirteenth trial on the day after transection. To minimize the confounding effects of learning on the results of the denervation experiments, we analyzed only feeding attempts that occurred soon after transection. Movements of the nostrils during breathing were also observed immediately after recovery from anesthesia in four toads that had received unilateral and/or bilateral denervation of the M. submentalis. After observation and video-taping of post-surgical feeding or breathing sequences, the toads were killed by overanesthesia in MS222. Denervation was confirmed by dissection, after which the toads were fixed in 10% formalin and preserved in 70% ethanol.

Kinematic analysis

The toads were video-taped while feeding under several conditions: before surgery ($N=7$ toads), after unilateral ($N=1$ toad) or bilateral ($N=3$ toads) denervation of the M. submentalis, and after unilateral ($N=1$ toad) or bilateral ($N=3$ toads) denervation of the Mm. genioglossus basalis and medialis (Table 1). For each individual and each treatment, three feeding sequences were digitized for kinematic analysis, with two exceptions (Table 1): (1) for one toad, three feeding sequences were digitized before surgery, but only one feeding sequence was obtained after bilateral denervation of the M. submentalis; and (2) for a second toad, three feeding sequences were digitized after unilateral denervation of the M. genioglossus, but only two sequences (one successful and one unsuccessful) were obtained before surgery. Successful and unsuccessful feeding sequences were observed and/or video-taped for several toads both before and after surgery. All digitized feeding sequences before surgery were successful captures, except one (Table 1). All digitized feeding sequences after unilateral and bilateral denervation of the M. submentalis were successful captures, whereas all digitized feeding sequences after unilateral and bilateral denervation of the Mm. genioglossus were unsuccessful capture attempts (Table 1).

Feeding behavior was video-taped at room temperature (21–25 °C). Waxworms (*Galleria* sp.) were used as prey and were placed at a distance of approximately 5 cm from the toads, in such a way that the prey moved directly toward the toads. The toads oriented toward the prey and initiated prey capture when the prey moved within striking distance. A Display Integration Technologies model DIT 660 high-speed, multi-framing video camera was used to film the toads at

Fig. 2. Camera lucida drawings of the peripheral nerves in the lower jaw (A) and tongue (B) of an adult *Bufo marinus* stained with Sudan Black B (jaw width 36 mm). (A) The trigeminal and glossopharyngeal nerves are shown on the left, and the facial and hypoglossal nerves are shown on the right. The stippled area indicates the tongue pad. On the left side, the trigeminal nerve is shown crossing over the mandible and innervates the M. intermandibularis and M. submentalis. The open arrow indicates the point at which the trigeminal nerve was transected for M. submentalis denervation. The glossopharyngeal nerve provides only sensory innervation of the tongue. On the right side, the facial nerve is shown innervating the M. interhyoideus at the base of the mandible. The hypoglossal nerve innervates the Mm. genioglossus basalis and medialis. The filled arrow indicates where the hypoglossal nerve was transected for Mm. genioglossus denervation. (B) Innervation of the tongue by the glossopharyngeal nerve (left) and hypoglossal nerve (right). The M. genioglossus basalis is innervated by the most proximal branches of the hypoglossal nerve at the base of the tongue, whereas the M. genioglossus medialis is innervated by the more distal branches.



120 fields s^{-1} with synchronized stroboscopic illumination at a resolution of 13.0–26.3 pixels cm^{-1} . Kinematic analysis of feeding behavior follows Nishikawa and Roth (1991) and Deban and Nishikawa (1992), except where otherwise noted. In each video field, the x,y -coordinates of the prey item, a nonmoving reference point and five points on the head of the toads were digitized directly from the video monitor. The digitized points were as follows: (1) tip of the upper jaw; (2) jaw joint; (3) midpoint of the mandible; (4) tip of the mandible; and (5) leading edge of the tongue. It is impossible to digitize the position of the lingual tip in every picture because it is obscured by the partly opened jaws in the earliest two or three fields of tongue protraction. Thus, we digitized the leading edge of the tongue (i.e. the most anterior point on the tongue) in each video field. Because the anatomical location of the leading edge of the tongue changes over time during protraction, the velocities and accelerations of the leading edge of the tongue underestimate the velocities and accelerations of the lingual tip. Because of the way that the tongue unfolds during protraction, the trajectories of the leading edge of the tongue and the tongue tip are nearly identical.

The durations (in milliseconds) of the following variables were measured from video tapes using Peak Performance Technologies motion-analysis software: (1) duration of approach, the time from the onset to the completion of forward movement of the upper jaw tip; (2) duration of mouth opening, the time from the onset to the completion of mouth opening; (3) duration of tongue protraction, the time from the onset to the completion of tongue protraction; (4) duration of tongue at target, the time from prey contact to the onset of tongue retraction; (5) duration of tongue retraction, the time from the onset to the completion of tongue retraction; (6) duration of mouth closing, the time from the onset to the completion of mouth closing; (7) duration of body recovery, the time from maximum displacement of the upper jaw tip to the completion of mouth closing; and (8) duration of feeding sequence, the time from first forward movement to the completion of mouth closing. The following variables were also measured in each picture: (1) maximum gape angle; (2) minimum mandibular angle (the angle subtended by the jaw joint and the mandibular tip with the midpoint of the mandible at the vertex); (3) maximum gape distance; (4) maximum tongue reach; and (5) distance to prey at the onset of prey capture. Maximum tongue reach was measured as the distance between the lingual tip and the tip of the mandible in the field at which maximum tongue protraction occurred.

The kinematic data were analyzed using Statview II and SuperAnova for the Macintosh IICI computer. Analysis of variance (ANOVA) and t -tests ($\alpha=0.05$) were used to compare the kinematics of feeding behavior among treatments (i.e. before *versus* after denervation of the *M. submentalis* or *Mm. genioglossus*). Strategies for dealing with the unbalanced data are described in detail in the Results section below. Also, because the toads differ in body size (Table 1) and because body size has been shown to affect feeding kinematics in toads (O'Reilly *et al.* 1995), it was necessary to take body size into

account in the statistical comparisons. Strategies for dealing with body size are also described in detail below.

A statistical problem that we encountered concerns the difference between comparison-wide *versus* experiment-wide error. When multiple dependent variables are tested for a given experiment, the probability that a given test will be significant owing to chance alone (Type I error) increases with the number of tests performed, and this is usually taken into account by dividing α (in this case 0.05) by the number of tests performed (i.e. the Bonferroni correction). However, Rice (1989) notes that the Bonferroni correction is overly conservative and, if used, will greatly increase the chance of accepting the null hypothesis when it is really false (Type II error). Rice (1989) suggests the use of the sequential Bonferroni correction, which is less conservative than the strict Bonferroni correction.

In the present study, there is an additional problem with application of the Bonferroni correction. For denervation experiments, the alternative hypothesis is that some kinematic variables should respond to treatment, but others should be unaffected. For example, under the ballista hypothesis, denervation of the *M. submentalis* should affect tongue protraction and mandibular bending, but not approach, mouth opening, tongue retraction or body recovery. If we were to count all of the dependent variables tested against the alternative hypothesis in the Bonferroni correction, we would greatly inflate the chance of a Type II error. In fact, only those variables that were predicted to change should be counted against the hypothesis. In consultation with a statistician (V. Meretsky, personal communication), we chose to use a sequential Bonferroni test in which only those variables that were predicted to change under the alternative hypothesis were counted against it. If there was no *a priori* prediction about which variables should change, then a sequential Bonferroni test was performed in which all of the dependent variables included in the analysis were counted against the alternative hypothesis. For each experiment, the issue of comparison-wide *versus* experiment-wide error is discussed in the Results section below.

To improve our understanding of the neural control of prey capture, we analyzed the trajectories of head, jaw and tongue movements for one representative successful capture before surgery (toad 6). For this sequence, the displacements and trajectories of the upper jaw tip, the mandibular tip and the lingual tip relative to an external reference were calculated from the digitized points. Also calculated were the trajectories of the mandibular tip relative to the upper jaw tip and of the lingual tip relative to the mandibular tip. When measured in this way, the trajectories exhibit additivity. For example, the trajectory of the mandibular tip relative to an external reference is equal to the trajectory of the upper jaw tip relative to an external reference plus the trajectory of the mandibular tip relative to the upper jaw tip. Likewise, the trajectory of the lingual tip relative to an external reference is the sum of (1) the trajectory of the upper jaw tip relative to an external reference, (2) the trajectory of the mandibular tip relative to

the upper jaw tip and (3) the trajectory of the lingual tip relative to the mandibular tip.

In each sequential frame, the displacements of the following points were calculated between successive frames: (1) the upper jaw tip relative to the reference point; (2) the mandibular tip relative to the upper jaw tip; and (3) the lingual tip relative to the mandibular tip. The velocities and accelerations of each point were then estimated from the change in position and the change in velocity between successive frames.

In order to explore the precision of coordination between tongue and jaw movements, a simple matrix model based on the trajectory analysis was developed. In this model, the displacement of the lingual tip relative to the mandibular tip was added to the other two displacements (i.e. the displacement of the mandibular tip relative to the upper jaw tip and the displacement of the lingual tip relative to the mandibular tip) either one video field (8.3 ms) before they actually occurred or one field after they actually occurred. The resulting trajectories of the lingual tip were calculated relative to the reference point. This matrix model allowed us to evaluate the importance of relative timing of lingual and mandibular displacements in determining the lingual trajectory.

Results

Electromyography

When patch electrodes are applied to the intact M. submentalis, the muscle shows EMG activity during feeding ($N=2$ toads, with 10 and 2 sequences respectively). There are two distinct bursts of activity separated by a silent interval of 40–60 ms. The first burst lasts 40–60 ms and the second burst lasts 60–80 ms. The maximum amplitude averages 2.27 mV among trials. After denervation of the M. submentalis, a small signal is recorded from the patch electrode during swallowing. However, the signal consists of only a single burst with a duration of 100–160 ms and an average amplitude of 0.17 mV, less than 10% of the amplitude recorded during feeding in the intact toads. In addition, there is no signal in response to mechanical manipulation of the denervated M. submentalis.

Muscle stimulation

When the mouth is closed, even minimal stimulation (<1 V) of the M. submentalis completely closes the nostrils, which open immediately after stimulation ceases. When the voltage is increased (6 V), the nostrils close immediately and the mouth then opens approximately 1 s after cessation of stimulation. When the M. submentalis is stimulated with the mouth open, the base of the tongue swells but does not rotate about the symphysis, which appears to bend ventrally.

When the M. genioglossus medialis is stimulated with the mouth open, the tongue swells and shortens. However, the lingual mass is neither lifted nor rotated about the symphysis. Placement of one electrode closer to the dense mass of the muscle, rather than near the end, produces more effective stiffening, perhaps because this induces less co-stimulation of

the M. hyoglossus. When the Mm. submentalis and genioglossus are stimulated synchronously, the tongue swells, shifts anteriorly and rises above the floor of the mouth, but does not rotate forward about the mandibular symphysis.

When the tongue is at rest in the floor of the mouth, stimulation of the M. hyoglossus at its muscular attachment to the hyoid plate pulls the base of the tongue posteriorly in the plane of the mandible. When the tongue is pulled out of the mouth, thereby shifting the M. hyoglossus into an extended position, stimulation of the M. hyoglossus causes the tongue to return rapidly to the resting position.

Stimulation of the M. geniohyoideus near its attachment to the hyoid causes a tensing of the lingual base, coupled with a lifting of the soft tissues adjacent to the mandibular symphysis. In a few cases, this stimulation causes the soft tissues of the tongue to swell and to rotate very slightly over the symphysis. Apparently, the symphyseal attachment of the M. geniohyoideus lies near the dorsal edge of the symphysis, so that its contraction lifts the anterior part of the buccal floor.

Bilateral stimulation of the M. depressor mandibulae depresses the mandibles and tends to rotate them medially which, since the mandibles are curved, depresses the symphyseal region. However, the symphyseal depression thus produced is less than that observed during feeding. Stimulation of the M. adductor mandibulae causes the mouth to close by rapidly lifting the mandibles, but it does not rotate them.

Kinematics of normal prey capture

The following description of the kinematics of successful prey capture in unoperated adult *Bufo marinus* is based on a sample of seven adult individuals, with 1–3 feeding sequences per individual, for a total of 19 successful feeding sequences and one unsuccessful prey capture attempt (Tables 1, 2). All kinematic variables are reported as means ± 1 standard error of the mean (S.E.M.), measured relative to the onset of mouth opening (time=0).

Bufo marinus maintains a stable head and body position when approaching prey. The fore- and hindlimbs remain on the substratum and there is little ventroflexion of the head (Fig. 3). Forward movement toward the prey begins before the onset of mouth opening and is completed shortly after prey contact. The head is placed within 2 mm of its maximum forward position at the time of prey contact, which demonstrates that the head and tongue movements are precisely coordinated.

As the mouth opens, the mandible bends downward at the mentomeckelian joint from a resting angle of 180° (Fig. 3). The tongue becomes visible as the mouth is opened, first rising from the floor of the mouth, and then appearing to unroll into an elongate shape as it rotates about the mandibular symphysis (Fig. 3). At maximum protraction, the tongue extends 3.9 ± 0.2 cm on average, ranging from 2.3 cm in the smallest toad to 5.9 cm in the largest toad. The mean angle between the long axis of the tongue and the mandible is $167 \pm 3^\circ$. After reaching their maximum forward position, the head and body of the toad return to the resting position. The mean duration of prey capture is 340 ± 15 ms (Table 2).

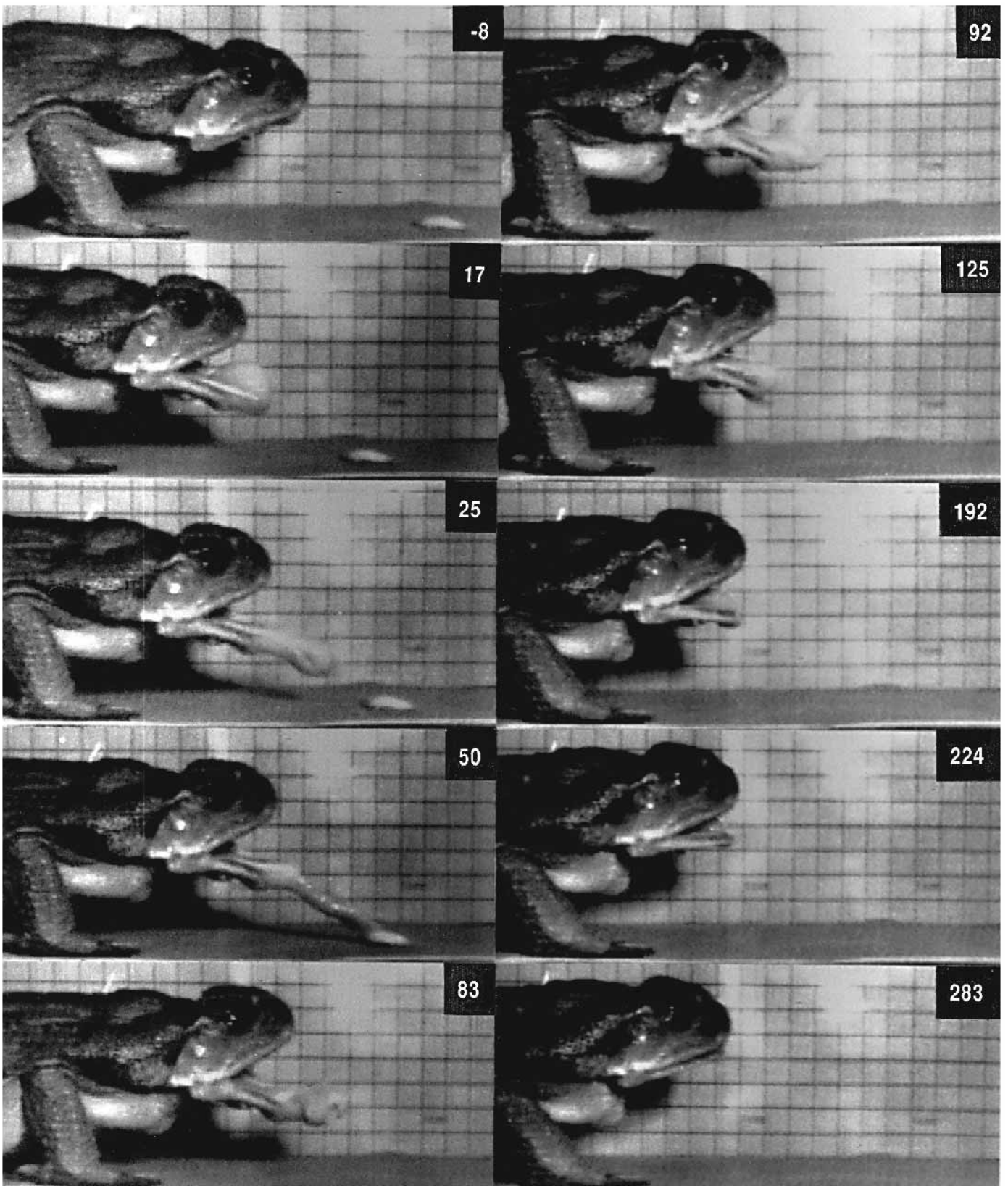


Fig. 3. Selected frames from a normal prey-capture sequence for *Bufo marinus* (toad 6) before surgery. The number in the top right-hand corner of each picture indicates the time (ms) from the onset of mouth opening. The squares in the background are 1 cm×1 cm. The tongue is protracted nearly 5 cm.

Table 2. Selected kinematic variables of prey capture attempts by *Bufo marinus*

Kinematic variable	Range	Mean	Miss	P-value
Duration of approach (ms)	125–258	179±9	200	0.2985
Duration of mouth opening (ms)	25–117	78±4	117	0.0308
Duration of tongue protraction (ms)	25–42	31±1	50	0.0005
Duration of tongue at target (ms)	8–25	17±1	–	–
Duration of tongue retraction (ms)	50–183	104±9	133	0.2390
Duration of mouth closing (ms)	58–250	129±10	108	0.3259
Duration of body recovery (ms)	100–300	161±11	183	0.3274
Duration of feeding sequence (ms)	250–491	340±15	383	0.2648
Maximum gape angle (degrees)	51–96	71±3.4	53	0.1340
Minimum mandibular angle (degrees)	132–154	145±1	138.6	0.1207
Maximum gape distance (cm)	2.7–4.1	3.3±0.1	2.9	0.1528
Maximum tongue reach (cm)	2.3–5.9	3.9±0.2	3.1	0.2007
Distance to prey (cm)	3.7–11.0	6.1±0.4	6.0	0.4948

Range and mean (\pm S.E.M.) values are given for successful prey captures before surgery ($N=7$ individuals with 1–3 sequences per individual, for a total of 19 successful feeding sequences) and for the single unsuccessful prey capture attempt (miss), together with the level of probability that it was drawn from the population of successful captures (t -tests, one-tailed, $\alpha=0.05/12=0.0042$).

In the single unsuccessful capture attempt, the toad failed to make contact with the prey. Because there was no *a priori* prediction about which variables should change, a sequential Bonferroni correction was used in which α was divided by the total number of dependent variables tested ($0.05/13=0.0038$). After applying the sequential Bonferroni correction, the only significant difference was that tongue protraction lasted longer in the unsuccessful capture attempt than in the successful ones ($P=0.0005$, Table 2).

During feeding, the trajectory of the lingual tip relative to an external reference follows a nearly straight line from the mouth to the prey (Fig. 4). The trajectory of the upper jaw tip relative to an external reference is downward and forward, then upward and backward (Fig. 4). The trajectory of the mandibular tip relative to that of the upper jaw is restricted to the vertical plane (Fig. 4). Relative to the mandibular tip, the lingual tip first rises as the tongue is protracted, but then changes direction abruptly, and moves downward and forward (Fig. 4). The straight-line trajectory of the lingual tip relative to an external reference is the sum of the other three trajectories.

Analysis of horizontal and vertical displacements and their first and second derivatives shows that the lingual tip reaches large accelerations relative to an external reference (maximum acceleration 310.35 m s^{-2} , more than 31 times the acceleration due to gravity) during tongue protraction. The acceleration of the tongue tip relative to the mandible is very similar (310.48 m s^{-2}). Although the acceleration is high, its duration is short (less than 8.3 ms). Thus, the lingual tip reaches a maximum resultant velocity of only 286 cm s^{-1} . Horizontal displacements of the upper jaw tip and mandibular tip are relatively small compared with that of the lingual tip, whereas vertical displacements of the mandibular and lingual tips are relatively large compared with that of the upper jaw tip (Fig. 5). The mandibular tip is displaced downward relative to the upper jaw tip at the same time as the lingual tip is displaced upward relative to the mandibles (Fig. 5).

The matrix model shows how small changes in the timing of mandibular and lingual displacements affect the trajectory of the lingual tip. Changes in timing of the displacements have a relatively small effect on the horizontal position of the tongue (Fig. 6). However, the vertical position of the lingual tip depends heavily upon the simultaneous displacement of the

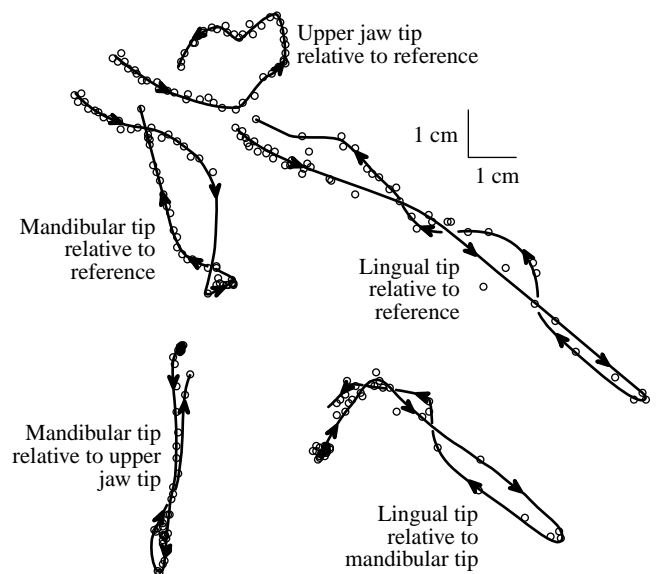


Fig. 4. Trajectories of the upper jaw tip relative to an external reference, the mandibular tip relative to an external reference, the lingual tip relative to an external reference, the mandibular tip relative to the upper jaw tip, and the lingual tip relative to the mandibular tip in *Bufo marinus*. Anterior is towards the right. All trajectories begin at the same point, but are offset for clarity. The arrows indicate the direction of movement in each segment of the trajectories, and the open circles indicate the position of each element in each video field. The lingual tip follows a nearly straight trajectory from mouth to prey during protraction. The trajectory of the mandibular tip relative to the upper jaw tip is largely limited to the vertical plane. The trajectory of the lingual tip relative to the mandibular tip is first upward and forward, but changes abruptly to downward and forward as the lingual tip passes beyond the mandibles.

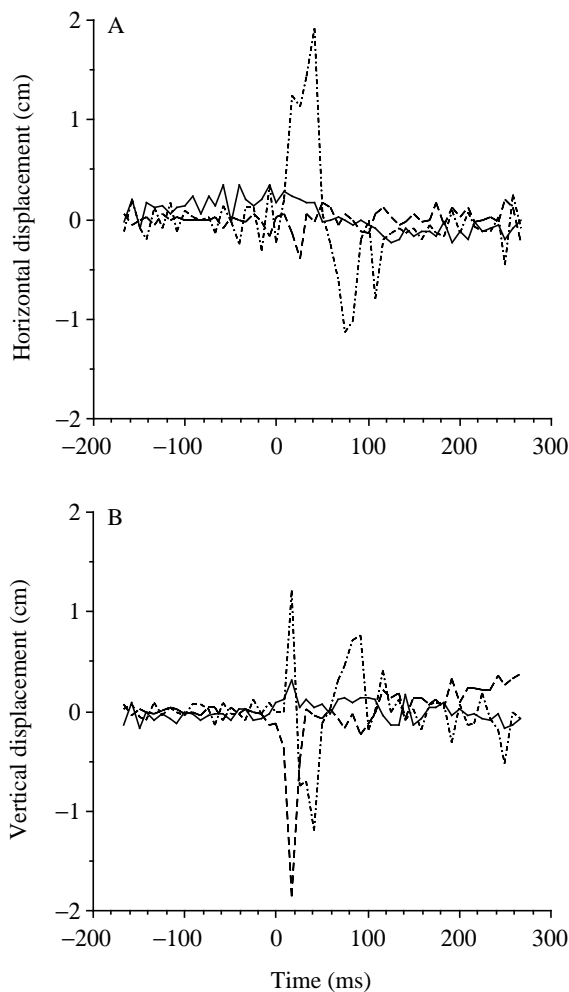


Fig. 5. Horizontal and vertical displacements between successive frames for the upper jaw tip (solid line), the mandibular tip (dashed line) and the lingual tip (dashed and dotted line) in *Bufo marinus*. (A) The horizontal displacements of the lingual tip are much greater than those of the upper jaw and mandibular tips and are positive during protraction and negative during retraction. (B) The downward displacement of the mandible during mouth opening occurs simultaneously with a large upward displacement of the lingual tip. Precise coordination of these tongue and jaw movements is necessary for a straight tongue trajectory (see Fig. 4).

mandibular and lingual tips (Fig. 6). The synchronization of these two accelerations is critical. If the vertical displacements of the lingual tip are added to the vertical displacements of the upper jaw and mandibular tips one video field (8.3 ms) earlier than they actually occur, then the trajectory of the lingual tip intersects that of the upper jaw (i.e. the tongue contacts the palate, Fig. 6). If the lingual displacements occur one field too late, then the lingual tip drops with the mandible before accelerating upward, which produces a non-linear trajectory that deviates from the observed trajectory (Fig. 6) by more than 1 cm (approximately 20% of tongue length or 27% of jaw length).

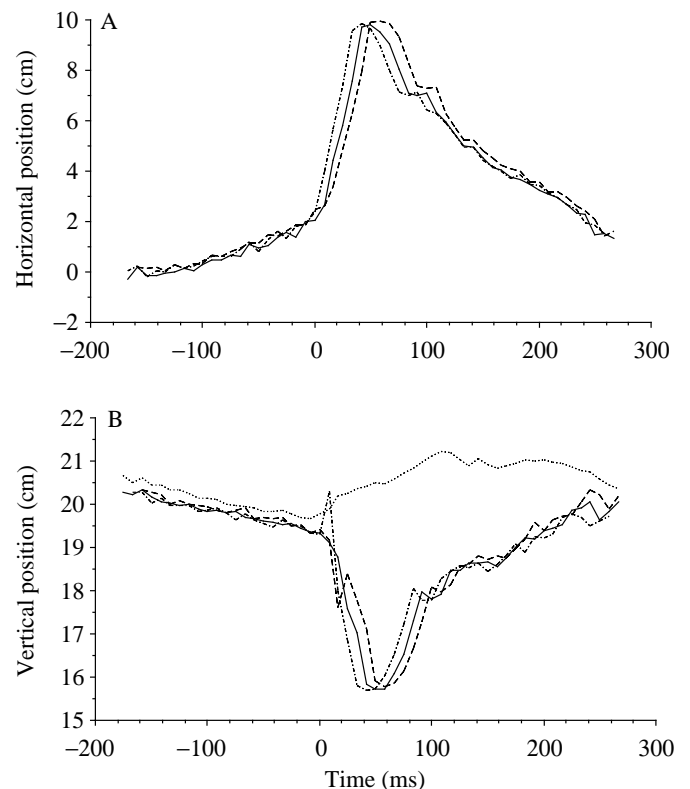


Fig. 6. A simple matrix model illustrating the consequences of small disruptions in the timing of jaw and tongue movements in *Bufo marinus*. The graphs show effects on (A) horizontal position and (B) vertical position. The solid line represents the change in position of the lingual tip relative to an external reference over time; the dashed line represents the changes in position that result if the tongue displacements are added to the upper jaw plus mandible displacements one video field (8.3 ms) later than they actually occur, and the dashed and dotted line represents the changes in position that result if the tongue displacements are added one field too early. If the tongue is accelerated too early, it contacts the palate (in the graph of vertical position, the palate is indicated by the higher dotted line). If the tongue is accelerated too late, its trajectory is not linear and deviates from the actual trajectory by more than 1 cm.

Denervation of the *M. submentalis*

Narial closure during breathing

Toads briefly close their nares once during every breathing cycle. They continue to breathe following unilateral ($N=1$) or bilateral ($N=3$) denervation of the *M. submentalis*. After unilateral denervation of the *M. submentalis*, bilateral narial movements are observed, but their amplitude is reduced so that the nares do not close completely. After bilateral denervation of the *M. submentalis*, the nares neither move nor close during breathing. These results demonstrate that contraction of the *M. submentalis* is necessary for narial closure during breathing. Lung ventilation can apparently occur in the absence of narial closure because the bilaterally denervated toads are able to breathe. However, casual observations after bilateral denervation indicate that the toads cannot inflate their lungs to distend the body as a defensive response during handling.

Thus, it appears that narial closure may be necessary for defensive inflation, although this question requires further study.

Prey capture

Digitized feeding sequences (Table 1) were compared among toads from three groups: pre-operative ($N=2$ toads with three sequences each); after unilateral denervation of the M. submentalis ($N=1$ toad with three feeding sequences); and after bilateral denervation of the M. submentalis ($N=3$ toads, with three sequences each for two individuals and one sequence for the third individual). The unbalanced design of this experiment made statistical comparisons among treatments difficult (Table 1). Two different statistical methods gave similar results. First, a one-way ANOVA was used to test for differences among the three treatments (Table 3). The mean value of each kinematic variable for each individual toad in each treatment was used (before, $N=2$; unilateral, $N=1$; and bilateral, $N=3$). This analysis did not take into account the fact that some individuals were used in more than one treatment or that body size differs among the toads (Table 1). However, it permitted simultaneous comparison among all three treatments. Second, each toad was compared with itself using t -tests (one-tailed, $\alpha=0.05$). This analysis takes into account body size differences among toads (because each individual is compared with itself) and the fact that some individuals were used in more than one treatment, but it does not allow simultaneous comparison of all treatments. The comparisons were as follows (Table 1): toad 2, unilateral ($n=3$) versus bilateral ($n=3$) denervation of the M. submentalis; toad 3, before ($n=3$) versus after ($n=1$) bilateral denervation of the M. submentalis; and toad 4, before ($n=3$) versus after ($n=3$) bilateral denervation of the M. submentalis.

The ballista hypothesis predicts that denervation of the M. submentalis should affect the duration of tongue protraction,

maximum tongue reach and minimum mandible angle, but the other kinematic variables should be unaffected. Thus, a sequential Bonferroni test was used in which $\alpha=0.05$ was divided by 3, yielding a corrected α -value of 0.0167. For both the ANOVA (Table 3) and the t -tests, none of the kinematic variables differed significantly among treatments. Both tests showed that neither unilateral nor bilateral denervation of the M. submentalis had any effect on maximum tongue reach or minimum mandibular angle during feeding (Table 3; Figs 7, 8).

Capture success was 66–100% before surgery and 50–100% after surgery. The difference was not significant for any individual toad, nor for the group as a whole (Fisher's exact probability test, $P>0.05$). In the feeding sequences after denervation of the M. submentalis, the tongue was always protracted successfully, even when the prey was missed.

Denervation of the Mm. genioglossus

The ballista hypothesis predicts that only the duration of tongue protraction and maximum tongue reach should be affected by denervation of the M. genioglossus. Thus, a corrected α -value of $0.05/2=0.025$ was used in the statistical tests. In order to study the effects of denervation of the M. genioglossus, we compared digitized feeding sequences before surgery ($N=4$ individuals, with 2–3 sequences per individual for a total of 11 sequences), after unilateral denervation of the Mm. genioglossus basalis and medialis ($N=1$ individual, with three feeding sequences) and after bilateral denervation of these muscles ($N=3$ individuals, with three sequences per individual). The data were analyzed in three ways. First, the feeding behavior of toad 5 was compared before and after unilateral denervation of the Mm. genioglossus using a t -test (one-tailed, $\alpha=0.025$). Second, a two-way ANOVA was used to compare before and after bilateral denervation of the Mm.

Table 3. Effects of M. submentalis denervation on feeding kinematics in Bufo marinus

Kinematic variables	Before	Unilateral	Bilateral	F-ratio	P-value
Duration of approach (ms)	164±14	178±18	193±13	2.010	0.2794
Duration of mouth opening (ms)	74±10	81±7	87±7	1.375	0.3769
Duration of tongue protraction (ms)	29±2	33±0	37±4	1.737	0.3154
Duration of tongue at target (ms)	19±2	19±3	17±2	1.112	0.4353
Duration of tongue retraction (ms)	115±16	119±16	117±23	0.003	0.9970
Duration of mouth closing (ms)	121±17	150±17	162±16	0.338	0.7373
Duration of body recovery (ms)	143±13	194±24	194±19	0.545	0.6283
Duration of feeding sequence (ms)	307±10	372±36	383±18	1.750	0.3136
Maximum gape angle (degrees)	72±8	75±5	70±3	0.008	0.9916
Minimum mandibular angle (degrees)	145±2	139±5	142±2	1.359	0.3800
Maximum gape distance (cm)	3.4±0.1	3.3±0.1	3.4±0.1	0.020	0.9799
Maximum tongue reach (cm)	3.7±0.4	3.6±0.2	3.7±0.4	0.022	0.9786
Distance to prey (cm)	5.5±0.5	4.3±0.4	5.2±1.0	0.102	0.9059

Means (\pm S.E.M.) of selected kinematic variables are given before surgery ($N=2$ individuals, six feeding sequences), and after unilateral ($N=1$ individual, three feeding sequences), and bilateral ($N=3$ individuals, seven feeding sequences) denervation of the M. submentalis. Only successful captures are included.

The F -ratios and P -values are for a one-way analysis of variance on individual means for each treatment (see text for further explanation).

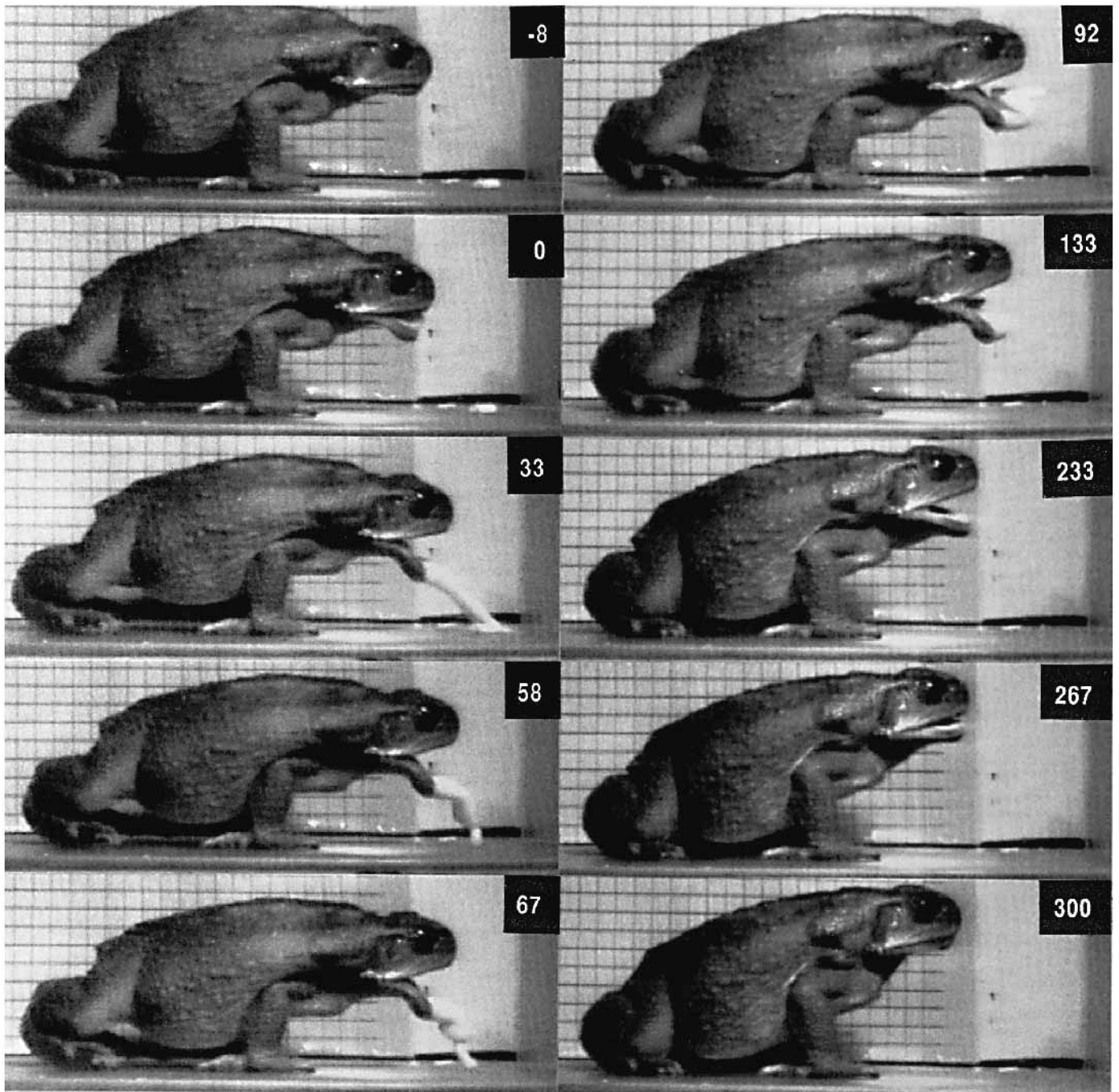


Fig. 7. Selected frames from a prey-capture sequence for *Bufo marinus* (toad 4) after bilateral denervation of the M. submentalis. Legend and scale as in Fig. 3. Note that the tongue is protracted and the mandible bends downward in a normal fashion.

genioglossus for toads 6, 7 and 8. For each two-way ANOVA, treatment (before *versus* bilateral denervation) was a fixed effect and individual was a random effect, so that the denominator of the F -ratio for treatment was the treatment \times individual mean square (Sokal and Rohlf, 1981). This analysis takes body size into account. Any effects that are due to body size will appear as a significant effect of individual in the two-way ANOVA. Third, a one-way ANOVA tested for differences among the three treatments (Table 4). The mean value of each

kinematic variable for each individual toad in each treatment was used (before, $N=4$; unilateral, $N=1$; and bilateral, $N=3$). As for the denervation of the M. submentalis, this analysis does not account for the fact that some individuals were used in more than one treatment (Table 1), but it permits a simultaneous comparison among all three treatments. Because similar results were obtained for the two-way and one-way ANOVAs, we present only the results of the one-way ANOVA (Table 4).

Table 4. *Effects of Mm. genioglossus denervation on feeding kinematics in Bufo marinus*

Kinematic variables	Before	Unilateral	Bilateral	F-ratio	P-value
Duration of approach (ms)	195±12	130±26	183±13	1.835	0.2525
Duration of mouth opening (ms)	82±6	97±3	47±12	2.120	0.2154
Duration of tongue protraction (ms)	33±2	33±0	25±6	0.632	0.5694
Duration of tongue retraction (ms)	107±13	128±3	90±7	0.517	0.6252
Duration of mouth closing (ms)	142±12	114±10	171±7	3.921	0.0946
Duration of body recovery (ms)	183±14	167±8	184±9	0.160	0.8567
Duration of feeding sequence (ms)	378±18	297±28	364±12	1.649	0.2818
Maximum gape angle (degrees)	64±3	50±1	80±3	4.260	0.0832
Minimum mandibular angle (degrees)	143±2	141±1	141±2	0.066	0.9366
Maximum gape distance (cm)	3.3±0.1	3.3±0.1	4.0±0.2	2.161	0.2107
Maximum tongue reach (cm)	4.2±0.3	2.1±0.1	1.2±0.2	8.794	0.0231
Distance to prey (cm)	6.5±0.7	3.9±0.2	6.1±0.7	0.610	0.5792

Means (\pm S.E.M.) of selected kinematic variables are given before ($N=4$ individuals, 2–3 sequences each, 11 sequences total), after unilateral ($N=1$ individual, three feeding sequences total) and after bilateral ($N=3$ individuals, three sequences each, nine sequences total) denervation of the *Mm. genioglossus* basalis and medialis. One unsuccessful and ten successful captures were included before surgery, and only unsuccessful captures were included after surgery.

The F -ratios and P -values are for a one-way analysis of variance on individual means for each treatment (see text for further explanation).

Comparison of the feeding behavior of toad 5 before *versus* after unilateral denervation of the *Mm. genioglossus* showed that the duration of tongue protraction ($P=0.0138$) was significantly shorter after unilateral denervation than before

denervation. Maximum tongue reach ($P=0.0069$) was significantly smaller after than before unilateral denervation. After unilateral denervation, the tongue showed no lateral deviation, either toward or away from the denervated side.

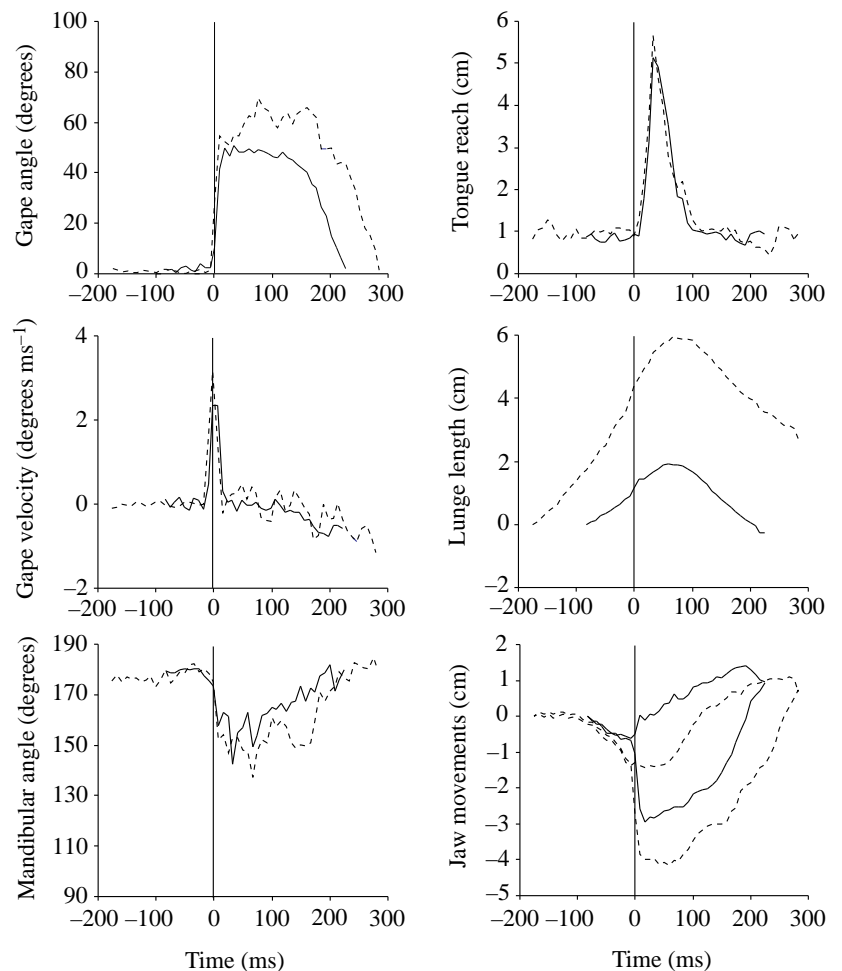


Fig. 8. Kinematic profiles of *Bufo marinus* (toad 4) before (solid lines) *versus* after (dashed lines) bilateral denervation of the *M. submentalalis*. Note the similarity in all of the kinematic variables, especially mandibular angle and tongue reach. Lunge length is the horizontal position of the upper jaw tip in each field. Jaw movements show the vertical position of the upper and lower jaw tips in each field.

The two-way ANOVA comparing feeding behavior before *versus* after bilateral denervation of the Mm. genioglossus for toads 6, 7 and 8 showed that maximum tongue reach was significantly shorter ($P=0.0140$) after bilateral denervation than before (Figs 9, 10). One-way ANOVA also showed a significant difference in maximum tongue reach among treatments ($P=0.0231$, Table 4).

Discussion

The mechanism of narial closure

On the basis of the observation that *Rana esculenta* lacks

striated narial muscles, Gaupp (1896a) proposed that anurans use an indirect method to close the nares during breathing. He observed that the nares close whenever a force applied to the tip of the premaxillae deforms the alary cartilages and he suggested that contraction of the M. submentalis should effect narial closure by lifting the mentomeckelian bones, which act indirectly to deform the alary cartilages. Shinkai and Narita (1957) demonstrated that the M. submentalis is active as intraoral and intrapulmonary pressures increase during breathing, and that there is a defect in nostril closure following denervation of the M. submentalis. Because this defect was not described in detail, the study failed to resolve whether the M.

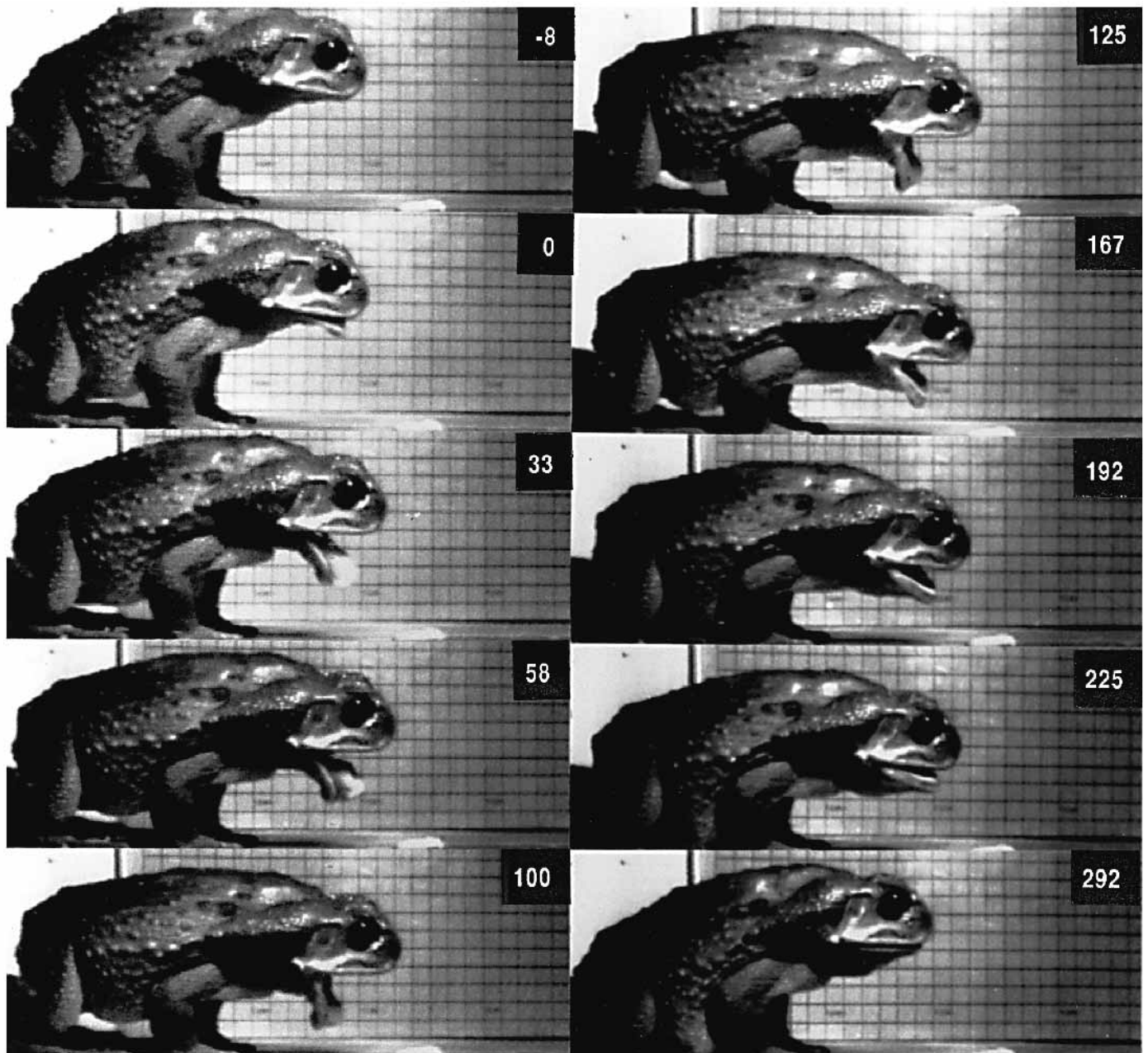


Fig. 9. Selected frames from a prey-capture sequence for *Bufo marinus* (toad 6) after bilateral denervation of the Mm. genioglossus. Legend and scale as in Fig. 3. Maximum tongue protraction occurs 58 ms after mouth opening and is reduced substantially compared with sequences before surgery (see Figs 3 and 10).

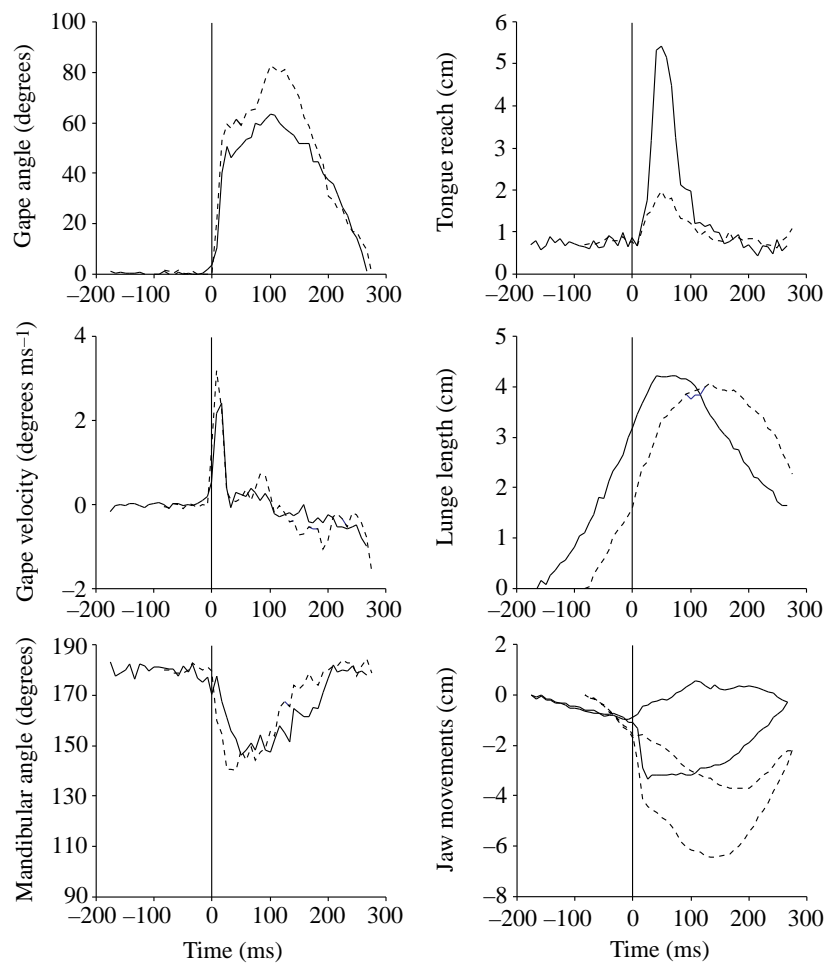


Fig. 10. Kinematic profiles before (solid lines) and after (dashed lines) bilateral denervation of the Mm. genioglossus basalis and medialis in *Bufo marinus* (toad 6). Note that the kinematic variables, except tongue reach, are similar before and after surgery. Tongue reach is reduced significantly after bilateral denervation of the Mm. genioglossus basalis and medialis.

submentalis alone is necessary or sufficient for narial closure. A later report (de Jongh and Gans, 1969) cited evidence from electromyography and muscle stimulation of intact frogs that confirmed Gaupp's explanation. However, various reports in the literature (e.g. West and Jones, 1975) persisted in referring to (nonexistent) striated muscles in the narial region. This led to an anatomical survey of more than 40 species of anurans and to EMG recordings in *Bufo marinus*, both of which further confirmed Gaupp's mechanism (Gans and Pyles, 1983).

In the present study, observations of video tapes showing narial movements following unilateral and bilateral denervation of the M. submentalis demonstrate that this muscle is necessary for narial closure. Bilateral denervation of the muscle completely abolishes closure of both nares, whereas unilateral denervation results in bilateral narial movements of reduced amplitude. These results are consistent with Gaupp's (1896a) model of narial closure.

Our observations also suggest that, whereas narial closure may increase the effectiveness of the buccal pump, it is not necessary for ventilation. Toads with bilateral denervation lack narial movement, but continue to inflate the lungs at least partially; they also survive for extended periods. If the mouth is artificially prevented from closing, toads continue to inflate their lungs although energetic studies suggest that the method

is costly (Bentley and Shield, 1973). Also, toads suffering from maggot infections that destroy the nostrils may survive for several weeks (Fischer, 1932). In contrast to ventilation, our observations suggest that narial closure may be necessary for defensive inflation.

The mechanism of tongue protraction

Gans and Gorniak (1982a,b) applied the ballista model of tongue protraction to toads (*Bufo marinus*) using electromyographic studies. In the ballista model, the Mm. genioglossus basalis and medialis and the M. submentalis form a lever system that flips the tongue over the mandibular symphysis during feeding in much the same way that a medieval ballista or trebuchet hurled missiles at the enemy (Tarver, 1995). In this model, the M. genioglossus medialis forms a stiffened rod, and the M. genioglossus basalis and M. submentalis together form a wedge that presses upward beneath the anteromedial end of the rod. The various depressors of the symphysis cause the mentomeckelian portion of the mandibles to bend ventrally, which further depresses the origin of the stiffened M. genioglossus medialis.

The results of the present study, based on kinematic analyses, muscle denervation and stimulation experiments, are in general concordance with previous studies of the feeding

system based on electromyography (Gans and Gorniak, 1982*a,b*) and video motion-analysis (Lauder and Reilly, 1994). However, the emphasis of the present study on the kinematic and dynamic aspects of tongue and jaw movement permits refinement of the prior scheme. Specifically, the results of the present study demonstrate that the posited ballista action (Regal and Gans, 1976) is by itself insufficient to lift and accelerate the soft tissues of the tongue. The mechanism of tongue protraction is more complex and involves additional aspects, which are described below.

The complexity of tongue and jaw movements during feeding is best demonstrated by examination of the trajectories of the upper jaw tip, mandibular tip and lingual tip (Fig. 4), which shows how the individual movements of these elements contribute to tongue protraction. A remarkable feature emerging from this analysis is that the lingual tip follows an almost straight line from mouth to prey, despite (1) the rotation of the tongue over the mandibular symphysis during protraction, from top to bottom as well as rear to front, and (2) the substantial change in the shape of the tongue during protraction (Fig. 3).

The straight trajectory of the lingual tip results from the combination of two partially opposed motions, that of the lingual tip relative to the mandibular tip and that of the mandibular tip relative to the upper jaw tip. Depression of the symphysis is a relatively simple motion that is confined almost entirely to the vertical plane (Fig. 4), whereas the movements of the lingual tip relative to the mandibular tip are rather complex. The lingual tip initially has an upward and forward trajectory relative to the tip of the mandibles, which changes abruptly to a downward and forward trajectory as the lingual tip passes beyond the oral cavity (Fig. 4). The tongue shortens during the early upward phase and elongates during the later downward phase of protraction. The initial trajectory is consistent with the ballista model of tongue protraction (Gans and Gorniak, 1982*a,b*). However, our results show that the later part of the trajectory requires simultaneous elongation of the tongue and depression of the mandibles.

A similar result was found in salamanders by Reilly and Lauder (1989). They showed that the forward movement of the tongue of *Ambystoma tigrinum* results from a balance of forces between muscles that produce dorsal and forward vectors (i.e. *Mm. intermandibularis*, *interhyoideus* and *subarcualis rectus*) and those that produce ventral and forward vectors (i.e. *M. geniohyoideus*). The result is that the tongue follows a straight trajectory from mouth to prey (Reilly and Lauder, 1989).

The role of the M. submentalis

In the ballista model, contraction of the *M. submentalis* is required to furnish a stiff, upward-pushing element at the base of the tongue and to depress the symphysis by pulling the mandibular tips together. However, kinematic analysis of feeding behavior before and after denervation of the *M. submentalis* indicates that its contraction is unnecessary for tongue protraction in *Bufo marinus*. Neither unilateral nor

bilateral denervation of the *M. submentalis* had any significant effect on tongue protraction (Table 3).

Gans and Gorniak (1982*a,b*) observed that the *M. submentalis* is active during feeding in intact toads (Gans and Gorniak, 1982*a,b*) and Matsushima *et al.* (1985) also observed that activity of the *M. submentalis* in toads when feeding was elicited by stimulation of the optic tectum. The electromyographic recordings made in the present study confirm these observations. The low level of activity recorded from the bilaterally denervated *M. submentalis* possibly represents cross talk from the *M. geniohyoideus*, which lies directly under the *M. submentalis* and has a single burst of activity during swallowing (Gans and Gorniak, 1982*a,b*). Also, no electrical event was observed upon mechanical stimulation of the *M. submentalis*. Thus, the EMG studies support the idea that the *M. submentalis* is active during feeding, but that its action is not required for tongue protraction.

Although *Bufo marinus* can still feed after denervation of the *M. submentalis*, the presence of the muscle alone may contribute to normal feeding. Denervation does not remove the mass of the muscle nor the effect of its presence on other muscles (e.g. *M. geniohyoideus* or *M. genioglossus medialis*). Similarly, the observation that a muscle contracts during the performance of a given type of behavior does not demonstrate that contraction of the muscle is necessary. For these reasons, biomechanical hypotheses of muscle function are best tested using a variety of techniques.

In *Bufo marinus*, as well as most other anuran species, movement of the mandibular symphysis relative to the cranium is complicated by the mobility of the intramandibular joint between the mentomeckelian bones and the dentary. Thus, depression of the mandibular tip results from a combination of downward movement of the mandibles relative to the cranium (mandibular depression) and downward movement of the mentomeckelian bones relative to the rest of the mandible (mandibular bending). In two anuran species, *Discoglossus pictus* (Nishikawa and Roth, 1991) and *Hyla cinerea* (Deban and Nishikawa, 1992), the *M. submentalis* is necessary for mandibular bending during feeding. In contrast, the mandibles are observed to bend downward substantially in *B. marinus* even after bilateral denervation of the *M. submentalis* (Table 3). The curvature of the mandibles during feeding seems to be affected by denervation of the *M. submentalis* (Fig. 7), but the change in curvature is too small to quantify statistically. In *B. marinus*, other muscles that insert on the mandibles (such as the *M. depressor mandibulae*, *M. intermandibularis* or *M. geniohyoideus*) seem to contribute to mandibular bending during feeding. This observation may explain why denervation of the *M. submentalis* does not eliminate mandibular bending in *B. marinus*.

The role of the Mm. genioglossus basalis and medialis

In the ballista model of tongue protraction (Gans and Gorniak, 1982*a,b*), the *M. genioglossus medialis* stiffens the tongue and the *M. genioglossus basalis* forms a wedge under the stiffened rod. After bilateral denervation of both muscles,

the tongue rises slightly from the floor of the mouth but no longer moves forward over the mandibles (Fig. 9). Thus, the Mm. genioglossus basalis and medialis are necessary for forward movement of the tongue. Unfortunately, it is very difficult to denervate the Mm. genioglossus basalis and medialis individually because the former is innervated by numerous small nerve branches near the mandibular symphysis (Fig. 2). Thus, it is beyond the scope of this study to distinguish the contributions of these muscles to tongue protraction.

Many anurans, including *Ascaphus truei*, *Discoglossus pictus* and *Hyla cinerea*, lack a M. genioglossus basalis (Horton, 1982). In both *Discoglossus* and *Hyla*, the M. genioglossus medialis is necessary for both tongue rotation and protraction (Nishikawa and Roth, 1991; Deban and Nishikawa, 1992). In these species, contraction of the M. genioglossus medialis raises the tongue pad and pulls it anteriorly, thereby rotating it forward about the symphysis, as proposed by Regal and Gans (1976). As the M. genioglossus medialis contracts in *B. marinus*, its distal fibers are pulled towards the center of the tongue pad. This shortening brings the lingual tip, as well as the center of the lingual mass, towards the symphysis. Thus, we hypothesize that contraction of the M. genioglossus medialis shortens and stiffens the tongue and pulls it forward towards the mandibular symphysis during the early phase of tongue protraction in *B. marinus* (Fig. 4).

As the mandibles are depressed, the inertia of the tongue lifts its soft tissues above the plane of the mandibles. Stimulation experiments suggest that contraction of the M. intermandibularis and/or M. geniohyoideus in the early stages of protraction may also contribute to the lifting of the tongue. It seems that the inertial lifting action resulting from the downward acceleration of the mandibles, combined with contraction of the M. intermandibularis and M. geniohyoideus, causes the initial upward trajectory of the lingual tip relative to the mandibular tip, whereas contraction of the M. genioglossus medialis is responsible for the initial forward movement of the lingual tip relative to the tip of the mandible.

In interpreting the muscular basis of tongue protraction in *Bufo marinus*, it is important to remember that, whereas the lingual pad contains only two pairs of extrinsic muscles (M. genioglossus medialis and M. hyoglossus), the lingual base is associated with several additional muscles including the M. genioglossus basalis, M. submentalis and M. geniohyoideus. These muscles lie deep to the soft tissues of the lingual pad and tip. Contraction of these muscles may contribute to the upward and forward movements of the tongue during protraction. In particular, the upward movement of the lingual tip may be enhanced by contraction of the M. geniohyoideus, which forms a platform and lifts the base of the tongue against the M. genioglossus basalis. Rotation of the tongue may be enhanced by depression of the symphyseal tip, to which the M. genioglossus medialis is attached. Whereas these muscles may enhance the initial forward and upward movements of the tongue, it appears from the denervation experiments that they are less important in force production than the accelerations of

the lingual tip that result from the pulling action of the Mm. genioglossus medialis and basalis.

At the inflection point in its trajectory (Fig. 4) between upward and downward movement, the acceleration of the lingual tip becomes negative. Thereafter, the lingual tip proceeds towards the prey powered by its own momentum, and eventually elongates by as much as 180% of its resting length. The lingual velocity decreases rapidly after the tip passes the inflection point. This is probably the result of internal friction, although activation of the M. hyoglossus might also exert a braking influence on the tongue pad. Because elongation of the tongue is inertial, the later phase of tongue protraction may be said to be projectile or ballistic.

As the lingual tip approaches the symphysis, the forward acceleration of the tongue decreases. Once the tongue pad has passed over the symphysis, continued contraction of the M. genioglossus medialis would impede tongue protraction by preventing relaxation and elongation of the tongue. Thus, the M. genioglossus medialis should cease contraction abruptly once the tongue has crossed the symphysis, so that the soft tissues of the tongue may continue forward passively under their own momentum. When the optic tectum of freely moving toads (*Bufo japonicus*) was stimulated to elicit feeding, electromyograms from the M. genioglossus medialis showed an abrupt cessation of activity at the appropriate time in the feeding cycle (Matsushima *et al.* 1985). This pattern is not evident in the analyses of Gans and Gorniak (1982a,b), but may have been obscured because they averaged EMG signals across both trials and individuals.

The peak upward acceleration of the lingual tip occurs simultaneously with the peak downward acceleration of the mandibles (Fig. 5). The matrix model (Fig. 6) demonstrates that the linear trajectory of the tongue depends upon these simultaneous accelerations. Apparently, the simultaneous acceleration balances opposing forces rather than transferring kinetic energy from the mandible to the tongue. The total acceleration of the lingual tip (310.35 m s^{-2}) is similar to its acceleration relative to the tips of the mandibles (310.48 m s^{-2}). The acceleration of the tip of the mandible relative to that of the upper jaw is oriented downward, whereas the acceleration of the lingual tip relative to that of the mandible is oriented upward and forward. Thus, the precise coordination of jaw and tongue movements seems to balance the partially opposed forces associated with their movements, thereby generating a straight lingual trajectory.

In a previous study (Nishikawa and Gans, 1992), we showed that the distal hypoglossal nerve of toads contains afferent fibers. Transection of the hypoglossal nerve alters the timing of activity in the M. levator mandibulae relative to that of the M. depressor mandibulae, so that these antagonists reach their peak activity simultaneously and the mouth fails to open. The hypoglossal afferents carry a signal from the tongue that modulates the activity of the jaw muscles. We suggest that these hypoglossal afferents provide a sensory signal that coordinates the simultaneous acceleration of the tongue and

mandible in the intact toad, thus ensuring that the lingual trajectory falls on the shortest path from mouth to prey.

Results from unilateral denervation of the Mm. genioglossus basalis and medialis also have interesting implications for the motor control of feeding behavior in *Bufo marinus*. It seems that sensory feedback from one side of the tongue is sufficient for mouth opening (Nishikawa and Gans, 1992). There are two alternative hypotheses that may explain why the mouth opens after unilateral hypoglossal transection: (1) sensory information may be transmitted to both sides of the brainstem simultaneously via axon collaterals, in which case the Mm. levator mandibulae would be inhibited on both sides; or (2) the sensory signal may only reach the M. levator mandibulae on the intact side, but inhibition of the M. levator mandibulae on that side may be sufficient for mouth opening, even though the muscle on the opposite side is not inhibited. The observation that unilateral hypoglossal transection reduces maximum gape angle and gape distance provides support for the idea that only one side of the Mm. levator mandibulae may be inhibited. This question could be resolved in future electromyographic studies.

Comparison with other anurans

Several features of oral anatomy appear to be similar across anuran species. Almost all anurans that have been studied have a tongue pad attached at the front of the mouth near the mandibular symphysis that is approximately as long as the mandibles when at rest in the floor of the mouth and is composed of two pairs of extrinsic muscles, the M. genioglossus medialis and the M. hyoglossus (Magimel-Pelonier, 1924; Regal and Gans, 1976; Horton, 1982).

However, despite these similarities, a dissertation (Magimel-Pelonier, 1924) first cited in 1976 was correct in noting that anurans display substantial diversity in lingual anatomy (Regal and Gans, 1976; Horton, 1982; Gans, 1982) and mechanisms of lingual projection (Trueb and Gans, 1983; Ritter and Nishikawa, 1995). Whereas the tongue of *Bufo marinus* shares the features listed above, the length of its tongue relative to the mandibles differs from that of basal frogs at full protraction. Among anurans, relative tongue length at maximum protraction varies from approximately 20% of mandibular length in *Discoglossus pictus* (family Discoglossidae; Nishikawa and Roth, 1991) to more than 300% in *Hemismus marmoratum* (family Hemisotidae; Ritter and Nishikawa, 1995). At 180% of mandibular length, the relative tongue length of *B. marinus* is second only to that of *H. marmoratum*.

Compared with frogs that possess short tongues (Nishikawa and Cannatella, 1991; Nishikawa and Roth, 1991; Deban and Nishikawa, 1992), the feeding behavior of *Bufo marinus* involves reduced head and body movements. In *B. marinus*, the limbs remain on the substratum during lingual projection, and the head rotates forward about the placement of the forelimbs. The head is positioned at snapping distance from the prey before the tongue is launched and moves forward only slightly after the onset of tongue protraction. In contrast, the short-tongued anurans accelerate the entire body so that all four limbs may leave the substratum (Nishikawa *et al.* 1992). These

frogs do not coordinate head and tongue movements as precisely as do toads and often overshoot prey by more than a centimeter. The long tongue of *B. marinus* may have the major advantage of permitting increased stability of the head during prey capture.

Another exceptional attribute of *Bufo marinus* is that it protracts its tongue more rapidly than any other species studied to date. The lingual tip of *B. marinus* reaches velocities of up to 286 cm s^{-1} and accelerations of more than 31 g during protraction. The velocity of the lingual tip in *B. marinus* is approximately 15–20 times faster than that of such frogs as *Hemismus marmoratum* (14 cm s^{-1} , Ritter and Nishikawa, 1995) or *Discoglossus pictus* (19 cm s^{-1} , Nishikawa and Roth, 1991). In contrast, movements of the body (approach and recovery) and jaws (mouth opening and closing) and retraction of the tongue are slower in *B. marinus* than in the other species. This is to be expected, given its much larger size.

The mechanism of tongue protraction in *Bufo marinus* shows both similarities with and differences from that of other frogs. The M. genioglossus medialis of *Discoglossus pictus* (Nishikawa and Roth, 1991) and *Hyla cinerea* (Deban and Nishikawa, 1992) rotates the tongue through an angle of less than 90° about the mandibular symphysis, and the trajectories of their lingual tips relative to those of the mandible are relatively simple. There is only one phase of tongue protraction; the lingual tip moves upward and forward while the tongue pad shortens. Unlike that of *B. marinus*, the lingual trajectory has neither an inflection point nor an inertial elongation phase. In contrast, the M. genioglossus medialis of *B. marinus* rotates through an angle of approximately 167° during protraction, and the trajectory of the lingual tip relative to that of the mandible is complex, as already described.

Nishikawa *et al.* (1992) conducted a phylogenetic analysis of 65 anuran species, representing 46 genera and 13 families. They found that several characteristics of prey capture represent the primitive condition among anurans, including a tongue that shortens during protraction, a mandible that bends downward, and forward lunging of the whole body. In contrast, a tongue that elongates during protraction and more stable head and body movements are derived within the Anura. On the basis of the pattern of similarities and differences in feeding kinematics and mechanisms of tongue protraction between *Bufo marinus* and other anurans, it seems that *B. marinus* has retained the plesiomorphic mechanism of tongue protraction found among archaeobatrachian frogs such as *Discoglossus pictus* (Nishikawa and Roth, 1991; Nishikawa *et al.* 1992). In these species, the M. genioglossus medialis pulls the lingual pad towards the mandibular symphysis, and the lingual tip displays an initial trajectory during which the tongue moves upward and forward relative to the tip of the mandibles as it shortens. To this plesiomorphic mechanism, several novel features have been added in *B. marinus*. The tongue has become longer (Nishikawa *et al.* 1992), it moves at greater velocities and accelerations during protraction, and the mechanism of tongue protraction includes a new ballistic phase

during which the tongue moves downward and forward as it elongates under its own momentum.

The ballistic phase of tongue protraction in *Bufo marinus* has several important implications for neural control. First, the transition to inertial elongation of the tongue greatly limits the degree to which its movements can be controlled by incoming sensory feedback after it has passed over the mandibular symphysis. In *Ascaphus truei*, *Discoglossus pictus* and *Hyla cinerea*, variability among trials is large even within individuals. If the tongue fails to contact the prey, then intraoral transport cycles may be inserted into a prey-capture sequence before the end of the first gape cycle (Nishikawa and Cannatella, 1991; Nishikawa and Roth, 1991; Deban and Nishikawa, 1992). Such modifications of the gape cycle never occur in *B. marinus*, presumably because feedback control can have no effect after the tongue has passed over the mandibular symphysis. Furthermore, differences in the kinematics of feeding movements between successful and unsuccessful capture attempts have been observed in several anuran species. In *Ascaphus truei* and *Discoglossus pictus*, successful and unsuccessful capture attempts differ only in movements that occur after prey contact (Nishikawa and Cannatella, 1991; Nishikawa and Roth, 1991), which suggests that these differences in movement patterns reflect sensory feedback resulting from prey contact. In contrast, the successful and unsuccessful capture attempts of *B. marinus* differ only in variables that occur before prey contact, which suggests that they are the result of a preprogrammed error, rather than of sensory feedback associated with prey contact. These lines of evidence suggest that the feeding behavior of *B. marinus* should be more stereotyped than that of anuran species with weakly protrusible tongues, such as *Ascaphus truei*, *Discoglossus pictus* and *Hyla cinerea*, because the inertial phase of tongue elongation in *B. marinus* is less susceptible to control by sensory feedback associated with prey contact. Of course, the lingual trajectory of *B. marinus* is still susceptible to feedforward control via modulation of the activity of either the M. genioglossus or M. hyoglossus.

In contrast, the hypoglossal afferents that coordinate movements of the jaws and tongue in *Bufo marinus* (Nishikawa and Gans, 1992) are absent in frogs with short tongues, including *Discoglossus pictus* (Nishikawa and Roth, 1991) and *Hyla cinerea* (Deban and Nishikawa, 1992). In addition, acceleration of the jaws and tongue does not occur simultaneously in these species as it does in *B. marinus*. Instead, their tongues experience much smaller displacements that occur 20–40 ms after the downward displacement of the mandibles. We hypothesize that the evolution of an inertial phase of tongue protraction in *B. marinus* has led to the evolution of novel hypoglossal afferents that coordinate the simultaneous acceleration of the tongue and jaws early in the feeding cycle.

The results of this study suggest that a relatively small change in the morphology of the feeding apparatus in *B. marinus* (i.e. elongation of the tongue) may have had important biomechanical consequences leading to the addition of a new,

inertial elongation phase to the tongue protraction mechanism. The biomechanical change in turn appears to have resulted in relatively large changes in neuromuscular control of the feeding apparatus, including the evolution of a novel afferent pathway for coordinating tongue and jaw movements, as well as increased stereotypy of the tongue flip relative to that of other frogs. Additional studies are necessary to test these hypotheses. Given the morphological diversity of the feeding apparatus among frogs, it is likely that further differences in neural control remain to be discovered.

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