

## THE MECHANISM OF TONGUE PROTRACTION DURING PREY CAPTURE IN THE FROG *DISCOGLOSSUS PICTUS*

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### Summary

The mechanism of tongue protraction in the archaeobatrachian frog *Discoglossus pictus* was studied using high-speed video motion analysis before and after denervation of the submental and genioglossus muscles. The kinematics of prey capture were compared (1) between successful and unsuccessful feeding attempts before surgery; (2) before and after denervation of the m. submental; and (3) before and after denervation of the m. genioglossus. Prey capture by *D. pictus* is similar to that of *Ascaphus truei*, hypothesized to be the sister group of all other living frogs. These archaeobatrachians have tongues of limited protrusibility (maximum tongue reach=0.21–0.27 cm) and lunge forward with the whole body to catch prey. In *Discoglossus*, unsuccessful attempts to capture prey differ from successful captures in having a longer duration of most kinematic variables. These results suggest that kinematic events are postponed in unsuccessful attempts at prey capture, owing to the absence of the somatosensory feedback that results from successful prey contact. Denervation of the m. submental prevents mandibular bending, but does not affect tongue protraction. Denervation of the m. genioglossus significantly decreases maximum tongue reach and maximum tongue height, but does not affect mandibular bending. The m. submental is necessary for mandibular bending, but neither mandibular bending nor m. submental activity are necessary or sufficient for tongue protraction. The m. genioglossus is necessary for normal tongue protraction. It does more than stiffen and support the tongue. These results are not consistent with the current model of tongue protraction developed for the neobatrachian toad *Bufo marinus*. If this model withstands the denervation test in *Bufo marinus*, then archaeobatrachians and neobatrachians must differ in their mechanisms of tongue protraction.

### Introduction

The most common methods that functional morphologists use to determine

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muscle function are (1) to describe the origin and insertion of muscle fibers, from which the direction of contraction can be determined (e.g. Regal and Gans, 1976), sometimes accompanied by electrical stimulation of muscles in an anesthetized animal (e.g. Trueb and Gans, 1983); and (2) to correlate the time of muscle activity with the occurrence of a behavioral event (e.g. Gans and Gorniak, 1982*a,b*; Matsushima *et al.* 1985). For some muscle systems, these methods may lead to robust hypotheses of function. However, there are other musculoskeletal systems for which these methods are less reliable, particularly those in which many muscles are involved, or in which many muscles are active simultaneously. The tongue protraction mechanism of frogs is a system that possesses both of these characteristics (Gans and Gorniak, 1982*a,b*; Matsushima *et al.* 1985).

Previous anatomical and electromyographic studies have identified several muscles believed to play roles in tongue protraction in frogs, including the m. submentalis, the mm. genioglossus basalis and medialis and the mm. geniohyoideus medialis and lateralis (Gans and Gorniak, 1982*a,b*; Emerson, 1977). The currently accepted biomechanical model of tongue protraction in frogs was developed from electromyographic analyses for the marine toad *Bufo marinus* (Gans and Gorniak, 1982*a,b*). In this model, the tongue is stiffened and supported by the m. genioglossus medialis. The m. genioglossus basalis provides a wedge under the anterior tip of the stiffened tongue. The m. submentalis acts on the wedge of the m. genioglossus basalis to raise and rotate the stiffened tongue over the mandibular symphysis, and bends the mandibles downward, which may also contribute to tongue protraction (Gans and Gorniak, 1982*a,b*). Thus, according to Gans and Gorniak's (1982*a,b*) model, contraction of both m. submentalis and m. genioglossus basalis should be necessary for normal tongue protraction, and contraction of the m. genioglossus medialis should be necessary for tongue stiffening.

An infrequently used technique for studying muscle function involves transection of the nerve branches innervating a particular muscle, in conjunction with studies of kinematics and/or electromyography before and after nerve transection (Nishikawa *et al.* 1989). These methods permit a test of the hypothesis that a particular muscle is either necessary or sufficient for the performance of a given motor behavior. A muscle is necessary for a given movement if denervation of that muscle alters the kinematics of the movement or eliminates the movement entirely. A muscle is sufficient for a given movement if denervation of other relevant muscles has no effect on the kinematics of the movement. Tests of these hypotheses are best made when the motor nerves that are transected contain no sensory fibers. In such cases, kinematic differences can be attributed directly to the absence of muscle activity. When the transected nerve also contains sensory fibers, an alternative interpretation, that the results are due to the disruption of sensory feedback, must also be considered.

The goal of this research was to study the feeding behavior of *Discoglossus pictus*, an archaeobatrachian frog with a feeding apparatus that represents the primitive condition for the order Anura (Magimel-Pelonnier, 1924; Regal and

Gans, 1976). We test the hypotheses that the *m. submentalis* and the *m. genioglossus medialis* are necessary protractors of the tongue in *Discoglossus*. The *m. genioglossus basalis* is absent from this species (Cannatella, 1985). This study is part of a phylogenetic survey of feeding mechanisms in diverse lineages of anurans.

### Materials and methods

Adult *Discoglossus pictus* Otth were obtained from animal suppliers and were bred in captivity at the Brain Research Institute, University of Bremen, FRG. The nine individuals used in this study were laboratory-reared subadults (i.e. sexually immature individuals, snout-vent length=32–41 mm). Feeding sequences were filmed between 20 and 22 November, 1988. Several *D. pictus* were cleared and the peripheral nerves were stained with Sudan Black B following the methods of Nishikawa (1987).

Individuals were videotaped while feeding before and after denervation of either the *m. submentalis* or the *m. genioglossus*. These muscles were denervated by surgical transection of peripheral nerves, near the point where the nerve branch enters the muscle (Fig. 1). The *ramus mandibularis* of the trigeminal nerve innervates the *m. submentalis* and contains both sensory and motor fibers (Gaupp, 1896; K. C. Nishikawa and G. Roth, personal observation). The *ramus hypoglossus* of the hypoglossal nerve innervates the *m. genioglossus* and also contains both

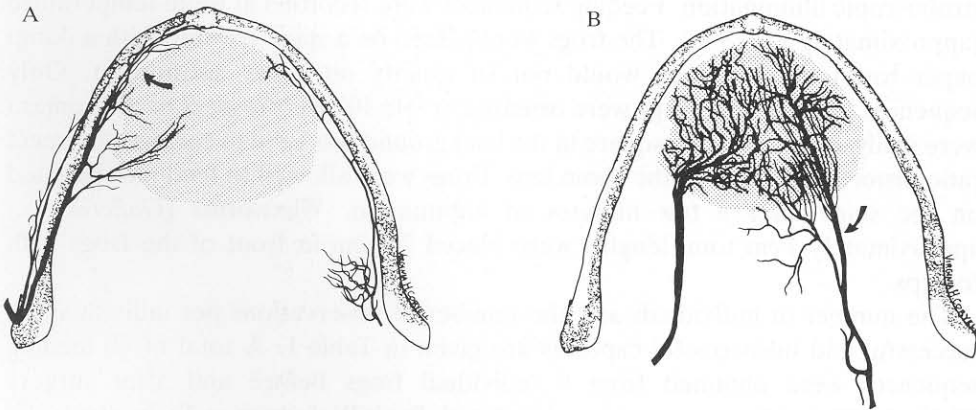


Fig. 1. *Camera lucida* drawing of the lower jaw of *Discoglossus pictus* stained with Sudan Black B. (A) Left, the *ramus mandibularis* of the trigeminal nerve. The most proximal branch innervates the skin of the lower jaw (sensory), the middle branch innervates the *m. intermandibularis posterior* and the most distal branch innervates the *m. submentalis*. The arrow indicates site of denervation of the *m. submentalis*. Right, the *ramus jugularis* of the facial nerve (sensory and motor). (B) Left, the *ramus lingualis* of the glossopharyngeal nerve (sensory only). Right, the *ramus hypoglossus* of the second spinal nerve. The branches, from proximal to distal, respectively, innervate the *mm. sternohyoideus*, *hyoglossus*, *geniohyoideus* and *genioglossus*. The arrow indicates site of denervation of the *m. genioglossus*. Jaw width=12 mm.

sensory and motor fibers (Stuesse *et al.* 1983). The frogs were anesthetized by immersion in 1.0% tricaine methanesulfonate (MS 222), after which branches of peripheral nerves were exposed and dissected free from surrounding connective tissue. A 2 mm length of the nerve was excised to retard regeneration.

The frogs recovered from anesthesia within approximately 30 min of surgery and began feeding immediately. The first feeding attempts of each frog following surgery were videotaped and analysed, so that learned adjustments of feeding behavior following surgery could be minimized. After post-surgical feeding attempts had been recorded, the frogs were killed by over-anesthesia in MS 222, and the location of nerve transection was confirmed by dissection.

A control treatment, in which some frogs receive anesthesia and surgical exposure of nerves without nerve transection, should be performed to ensure that the effects of surgery are not artifacts of anesthesia or of tissue disruption during surgery. This was not possible, however, because of the limited number of specimens of *D. pictus* available for study. Fortunately, however, the two different surgical treatments can partially serve as each other's controls. Differences between the two surgery groups cannot be attributed to anesthesia or to general effects of tissue disruption because these are the same in both treatments. Thus, general effects of surgery and anesthesia are only confounded with specific effects of denervation for variables that are similar in both of the post-surgical treatments.

A Display Integration Technologies<sup>TM</sup> model DIT 660 high-speed, multi-framing video camera was used to film the frogs at 180 fields s<sup>-1</sup> with synchronized stroboscopic illumination. Feeding sequences were recorded at room temperature (approximately 22–23°C). The frogs were placed on a stage covered with a damp paper towel because they would not sit quietly on a dry substratum. Only sequences in which the frogs were oriented at 90±10° with respect to the camera were analyzed. A 12.7 mm square in the background was used to correct for aspect ratio distortion caused by the zoom lens. Frogs were allowed to feed unrestrained on the stage after a few minutes of habituation. Waxworms (*Galleria* sp., approximately 1 cm total length) were placed 2–4 cm in front of the frogs with forceps.

The number of individuals and the number of observations per individual for successful and unsuccessful captures are given in Table 1. A total of 40 feeding sequences were obtained from 9 individual frogs before and after surgery (Table 1). The videotapes were analysed with Peak Performance Technologies<sup>TM</sup> 2D motion analysis software. The resolution of the video image was 40 pixels cm<sup>-1</sup>. High-frequency noise resulting from digitizing error was filtered from the data using a Butterworth digital filter. On each frame, the *x,y*-coordinates of the prey item, 10 points on the head of the frog (Fig. 2) and a non-moving reference point were digitized directly on the video monitor. At least 12 sequential frames were digitized for each sequence.

For the following events, the time at which each was observed relative to the onset of mouth opening ( $t=0$ ) was recorded: (1) onset of forward head movement; (2) onset of tongue protraction; (3) maximum mandibular bending; (4) prey

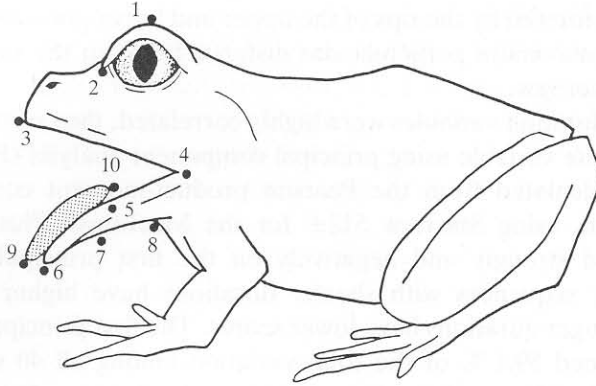


Fig. 2. Diagram of the 10 digitized points from which kinematic variables were derived. (1) Top of eye, (2) anterior corner of eye, (3) tip of upper jaw, (4) jaw joint, (5) midlength of lower jaw, (6) tip of lower jaw, (7) floor of mouth, (8) hyoid, (9) anterior tip of tongue and (10) posterior tip of tongue.

contact; (5) completion of tongue protraction; (6) maximum forward excursion of the head; (7) maximum gape; (8) onset of tongue retraction; (9) completion of tongue retraction; (10) onset of mouth closing; and (11) completion of mouth closing.

Duration variables were calculated from the onset and completion variables as follows: (1) duration of approach = maximum forward excursion - onset of forward head movement; (2) duration of mouth opening = maximum gape - onset of mouth opening; (3) duration of tongue protraction = completion of tongue protraction - onset of tongue protraction; (4) duration tongue remains at target = onset of tongue retraction - prey contact; (5) duration of tongue retraction = completion of tongue retraction - onset of tongue retraction; (6) duration of mouth closing = completion of mouth closing - maximum gape; (7) duration of body recovery = maximum forward excursion - completion of mouth closing; (8) duration of feeding sequence = completion of mouth closing - onset of forward head movement.

The following variables were calculated directly from the digitized points: (1) maximum gape angle = maximum angle subtended by the tips of the upper and lower jaws with the jaw joint at the vertex; (2) minimum angle of mandibular bending = minimum angle subtended by the jaw joint and the tip of the lower jaw with the midpoint of the lower jaw as the vertex (note: the mandible angle at rest is  $180^\circ$  and bends ventrally to an angle of about  $150^\circ$  during mouth opening); (3) maximum absolute gape = distance between tips of upper and lower jaws; (4) distance to prey = distance between tip of snout and prey before the onset of forward head movement; (5) lunge length = distance between tip of snout at rest and at maximum forward excursion; (6) overshoot distance = distance between tip of snout at time of prey contact and tip of snout at time of maximum forward excursion; (7) maximum tongue reach = maximum distance the tongue protrudes

beyond the line formed by the tips of the upper and lower jaws; and (8) maximum tongue height=maximum perpendicular distance between the top of the tongue pad and the lower jaw.

Because the duration variables were highly correlated, they were combined into a single composite variable using principal component analysis (PCA). The PCA solution was calculated from the Pearson product-moment correlation matrix without rotation, using Statview 512+ for the MacIntosh Plus computer. All variables loaded strongly and negatively on the first principal component of duration. Thus, sequences with shorter durations have higher scores and sequences with longer durations have lower scores. The first principal component of duration explained 59.1% of the total variation among all 40 sequences in all duration variables. For each feeding sequence, the duration factor score on the first principal component was calculated as the sum over all duration variables of the observed values of each variable weighted by the factor coefficient of that variable on the first principal component.

Tongue height and tongue reach were highly correlated, and were also combined into a first principal component of tongue protraction that maximized the explained variation in tongue reach and tongue height simultaneously. The first principal component of tongue protraction accounted for 85.4% of the variation among all 40 sequences in both tongue variables. The tongue factor score for each frog was calculated as the mean of the factor scores for all sequences for that frog before or after surgery. Both tongue variables loaded strongly and positively on the first principal component of tongue protraction. Thus, sequences with less tongue protraction have lower scores and sequences with more tongue protraction have higher scores.

Correlations between kinematic variables and lunge distance were calculated using Pearson's product-moment correlation coefficient. Differences between treatments in the probability of successful capture were analysed using Fisher's exact probability tests (Sokal and Rohlf, 1981).

Successful captures and unsuccessful attempts at prey capture before surgery were compared using one-way analysis of variance (ANOVA). Success is the fixed effect and the residual variance is between individuals within capture or miss. The degrees of freedom are given in Table 2. For each individual frog, the mean of successful or unsuccessful sequences, but not both, was used (see Table 2). The dependent variables are the factor scores on the first principal component of duration, the minimum mandible angle and the factor scores on the first principal component of tongue protraction (see Table 2). The significance of model effects is evaluated at  $P \leq 0.05$ .

Kinematic variables were compared before and after surgery using one-way analysis of variance. The dependent variables are the factor scores on the first principal component of duration, the minimum mandible angle and the factor scores on the first principal component of tongue protraction (see Tables 3 and 4). For each individual frog, the mean of all sequences, including both successful and unsuccessful capture attempts, was used. No individual was used in both

treatments (see Tables 3 and 4). Thus, the residual variance is between individuals within treatments. Because the successful and unsuccessful capture attempts of individual frogs were combined, success of capture is confounded with treatment effects for variables that differ between successful and unsuccessful capture attempts. However, neither minimum mandible angle nor the tongue protraction variables differed between successful and unsuccessful prey capture attempts (see Table 2). Only the analysis of duration variables is affected by this problem.

## Results

### *Kinematics of normal feeding behavior*

Prey capture involves movements of the body (approach and recovery), of the jaws (mouth opening and closing) and of the tongue (protraction and retraction). In *D. pictus*, the prey capture sequence is completed in 112–190 ms, depending on the length of the lunge, on the success or failure of the capture attempt, and on whether an intraoral transport cycle occurs before mouth closing. Fifteen of 24 attempts at capture (62.5%) were successful (Table 1). Unsuccessful capture attempts and sequences with long lunges or with intraoral transport cycles require more time for completion.

Duration of approach ( $r=0.552$ ), duration of mouth opening ( $r=0.561$ ), maximum absolute gape ( $r=0.639$ ), onset of mouth closing ( $r=0.534$ ), distance to prey ( $r=0.902$ ) and overshoot distance ( $r=0.584$ ) were significantly positively correlated with lunge distance (all  $P<0.05$ ). No other kinematic variables were significantly correlated with lunge distance (all  $P>0.05$ ).

### *Approach*

Prey capture begins as the frog's head moves towards the prey. Forward head movement begins on average 27 ms before the onset of mouth opening and usually ceases after maximum gape, simultaneously with or shortly before the onset of

Table 1. *Number of successful and unsuccessful capture attempts before and after surgery for the nine frogs included in this study*

	Individual								
	1	2	3	4	5	6	7	8	9
Before surgery									
Miss	1	0	2	0	0	0	0	3	3
Capture	2	3	1	3	2	2	2	0	0
After surgery									
Miss	3	0	3	—	3	2	—	3	—
Capture	0	1	0	—	0	1	—	0	—

Frogs 1, 2 and 3 received surgical denervation of the m. submentalis and frogs 5, 6 and 8 received surgical denervation of the m. genioglossus.

tongue retraction. The frogs reach their maximum forward excursion 85 ms after the onset of mouth opening.

### *Mouth opening*

As the mouth opens, the eyes are retracted into the orbits and the flexible mandible bends downwards at the mentomeckelian joint for the first time (Figs 3A, 4A). Maximum mandibular bending (mean  $\pm$  s.e. =  $150.3^\circ \pm 2.7$ ) occurs on average 27 ms after the onset of mouth opening. Mouth opening begins after

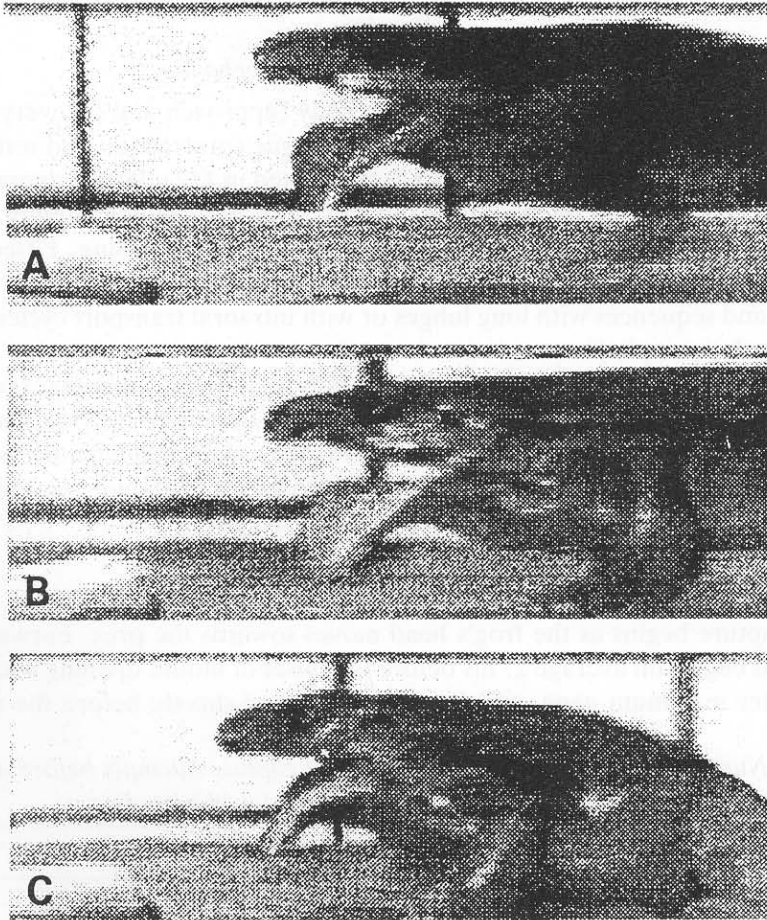


Fig. 3. Video images of *Discoglossus pictus* during feeding at maximum tongue protraction. (A) Normal feeding before surgery. The mandible bends downwards to an angle of about  $140^\circ$  and the tongue is protracted approximately 2 mm beyond the jaw tip. (B) After bilateral denervation of the m. submentalialis. The mandible does not bend downwards, but remains straight at an angle of about  $180^\circ$ , but the tongue is nevertheless protracted approximately 2 mm beyond the jaw tip. (C) After bilateral denervation of the m. genioglossus. The mandible bends downwards to an angle of about  $140^\circ$ , but the tongue is not elevated in the floor of the mouth and is not protracted beyond the mandible.



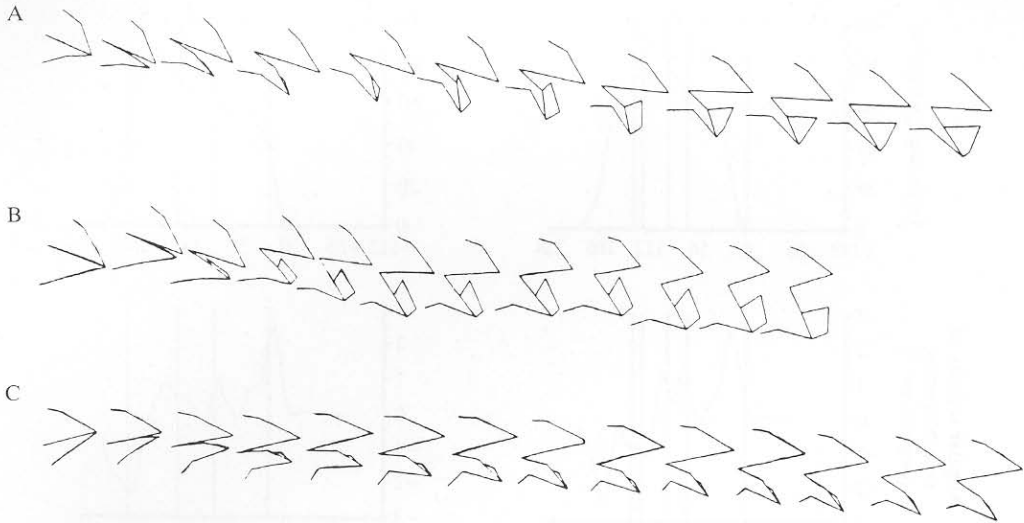


Fig. 4. Digitized images of the head during feeding, from the onset of mouth opening (first frame) to maximum tongue protraction (last frame). Every frame is included. (A) Normal feeding before surgery. The mandible bends downwards, reaching its minimum angle in frame 7. (B) After bilateral denervation of the *m. submentalialis*. The mandible angle is approximately  $180^\circ$  in all frames. (C) After bilateral denervation of the *m. genioglossus*. The mandible bends, reaching its minimum angle in frame 9, but the tongue is not elevated or protracted in any frame.

the onset of forward head movement and is completed after maximum tongue protraction, but before the onset of tongue retraction. In many cases, there is an indication of a slow-opening phase of mouth opening occurring after a faster-opening phase (Figs 5, 6), but in no case was there a slow-opening phase occurring before a fast-opening phase.

#### *Tongue protraction*

Shortly after (mean = 10.5 ms) the onset of mouth opening, the tongue appears as a relatively flat, broad pad that is raised slightly from the floor of the mouth. By the time of prey contact, the posterior tongue pad is elevated and the tongue has thickened and become more compact. The tongue reaches its maximum protraction on average 55 ms after the onset of mouth opening, shortly after prey contact. At maximum protraction, mean tongue reach is 2 mm and mean tongue height is 4 mm.

#### *Prey contact*

Prey contact occurs on average 44 ms after the onset of mouth opening and 12 ms before maximum tongue protraction. Maximum forward excursion occurs on average 41 ms after prey contact. The mean overshoot distance is 0.30 cm. The time from prey contact to the onset of tongue retraction ranges from 17 to 62 ms.

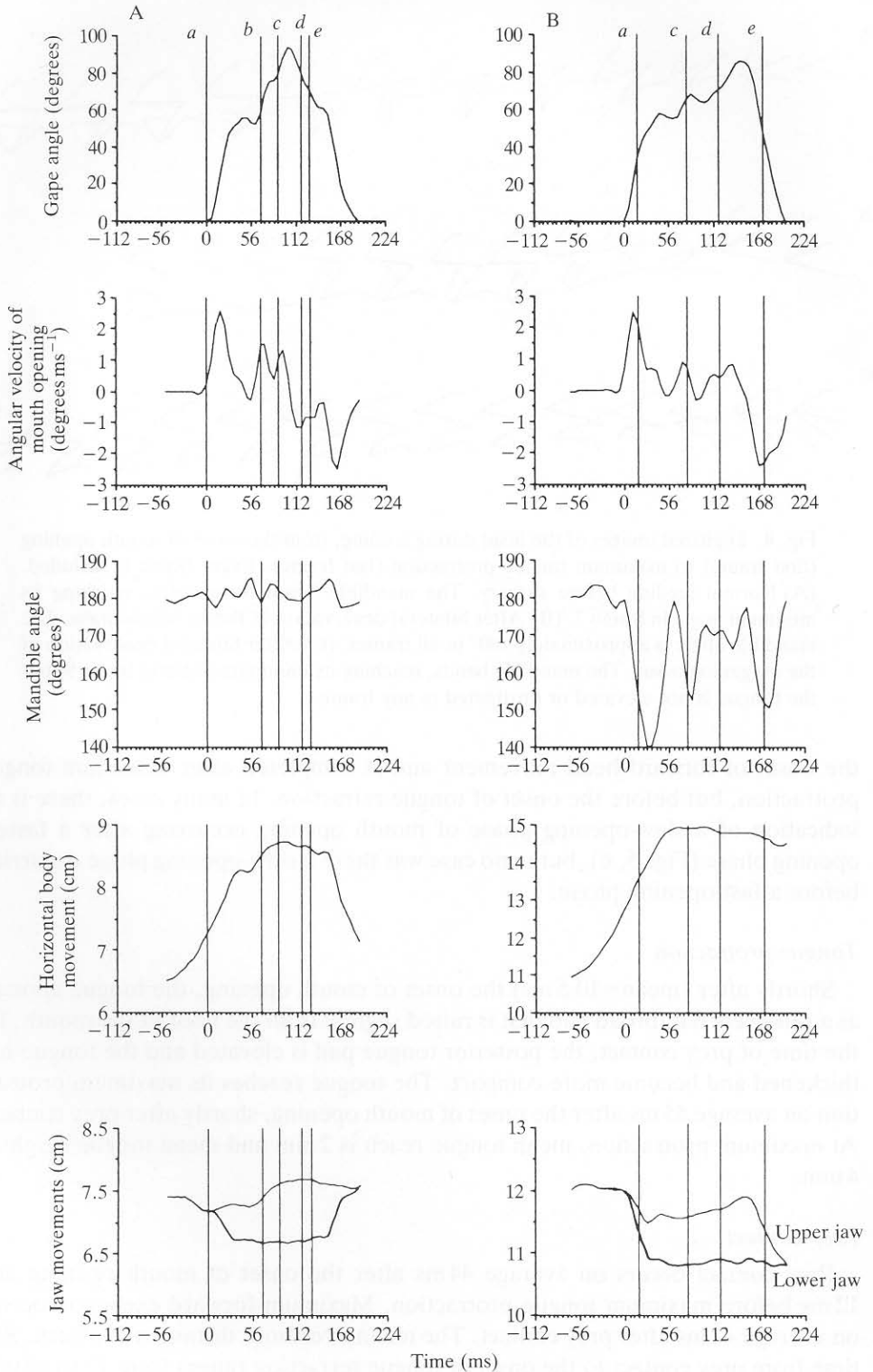


Fig. 5

Fig. 5. Kinematic variables. From upper to lower, respectively, gape angle, angular velocity of mouth opening, mandible angle, which during normal feeding is  $180^\circ$  at rest and decreases to a minimum of about  $140^\circ$  as the mouth opens, horizontal lunging movement of the body, vertical movements of the upper and lower jaws (the distance between the two lines is absolute gape). The vertical lines represent, from left to right, (a) onset of tongue protraction, (b) prey contact, (c) maximum tongue protraction, (d) onset of tongue retraction and (e) completion of tongue retraction. (A) After bilateral m. submentalis denervation. (B) The same individual before surgery. This was an unsuccessful feeding attempt, so there is no line representing the time of prey contact. A and B are similar, except that the mandible angle remains relatively stable near  $180^\circ$  after surgery.

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### *Maximum gape*

Maximum gape of the jaws occurs on average 77 ms after the onset of mouth opening. The mean maximum gape angle is  $81.6^\circ$  and the mean maximum absolute gape is 1.2 cm.

### *Body recovery*

During the body recovery phase, the frog returns to its resting position near its original location, the tongue retracts and the jaws close on the prey. Recovery begins shortly after the onset of tongue retraction and ceases with the completion of mouth closing. The mean duration of the body recovery phase is 49.7 ms.

### *Tongue retraction*

Tongue retraction begins after mouth opening and forward head movement have ceased, on average 86 ms after the onset of mouth opening, and is completed before mouth closing, on average 114 ms after the onset of mouth opening.

### *Mouth closing*

Mouth closing begins at the same time as the onset of tongue retraction, on average 84 ms after the onset of mouth opening, and ceases at the end of the prey capture sequence, on average 134 ms after the onset of mouth opening. Longer mouth closing times are associated with insertion of an intraoral transport sequence into the final stages of the prey capture sequence. The mean velocity of mouth closing ( $2.6^\circ \text{ms}^{-1}$ ) is similar to the velocity of mouth opening ( $2.5^\circ \text{ms}^{-1}$ ).

### *Comparison of successful and unsuccessful feeding attempts*

The results of the one-way analysis of variance comparing successful and unsuccessful feeding attempts before surgery are shown in Table 2. Factor scores on the first principal component of duration variables are significantly lower during successful captures than during unsuccessful attempts at capture ( $P < 0.025$ , Table 2). This means that the durations of kinematic variables were longer in unsuccessful capture attempts. Duration of mouth opening, duration of tongue retraction and duration of recovery are significantly longer in unsuccessful

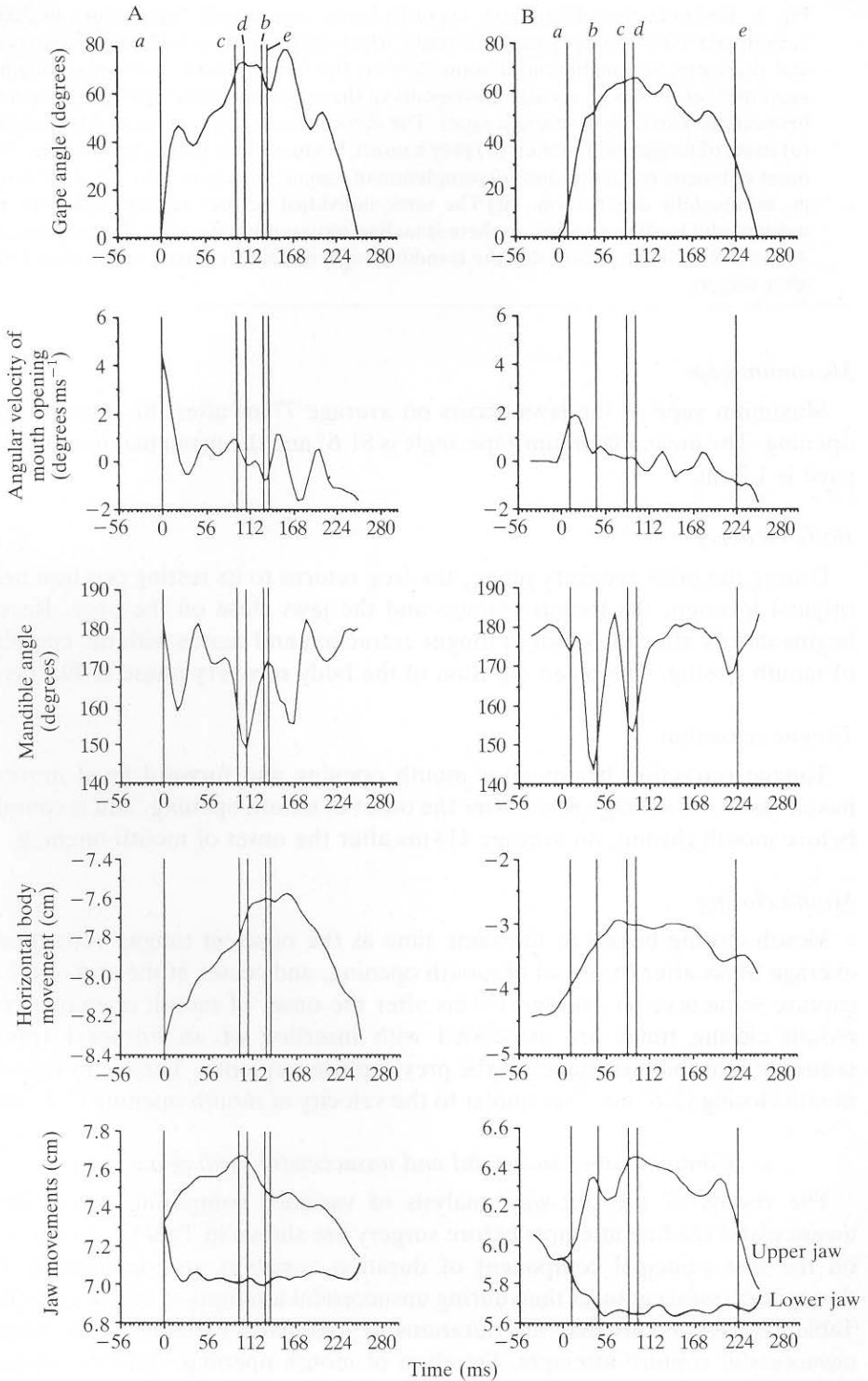


Fig. 6

Fig. 6. Details as for Fig. 5. (A) After bilateral *m. genioglossus* denervation. (B) The same individual before surgery. In A, after surgery, the order of the vertical lines is different. Prey contact (*b*) occurs later than maximum tongue protraction (*c*) and the onset of tongue retraction (*d*), but before the completion of tongue retraction (*e*). Otherwise, the graphs are similar.

attempts at capture, and contribute to the significance of the difference (all  $P < 0.05$ ). Neither the tongue protraction factor scores nor the minimum mandible angles differ significantly between successful and unsuccessful capture attempts (Table 2).

#### *Effects of bilateral m. submentalis denervation on feeding behavior*

The results of the one-way analysis of variance comparing feeding attempts before and after bilateral denervation of the *m. submentalis* are shown in Table 3. Neither the duration factor scores nor the tongue protraction factor scores differ significantly before and after surgery. Mandibular bending was reduced significantly by *m. submentalis* denervation ( $P < 0.025$ ), from  $154.6^\circ$  before surgery to  $174.6^\circ$  after surgery (Table 3, Figs 3B, 4B, 5). Capture success (16.7%) was significantly lower after denervation of the *m. submentalis* than before denervation (Fisher's exact probability test,  $P = 0.0349$ ).

#### *Effects of bilateral m. genioglossus denervation on feeding behavior*

The results of the one-way analysis of variance comparing feeding attempts before and after bilateral denervation of the *m. genioglossus* are shown in Table 4. Only minimum mandibular bending angle was unaffected by denervation of the *m. genioglossus*. As in unsuccessful attempts at capture, the duration factor scores are significantly lower after surgery than before surgery ( $P < 0.025$ , Table 4), which means that the durations of kinematic variables were longer after *m. genioglossus* denervation. Duration of mouth opening and closing, duration of tongue protraction and duration of recovery contribute to the significance of the difference (all  $P < 0.05$ ).

Table 2. *One-way analysis of variance with success (capture vs miss) as the fixed effect (d.f. = 1)*

Dependent variable	Capture Mean $\pm$ S.E.	Miss Mean $\pm$ S.E.	F-ratio Success
Duration factor	0.960 $\pm$ 0.173	-0.3568 $\pm$ 0.187	26.526*
Minimum mandible angle (degrees)	151.2 $\pm$ 3.4	149.7 $\pm$ 6.4	0.092
Tongue factor	0.509 $\pm$ 0.289	-0.087 $\pm$ 0.318	1.919

The residual variance (d.f. = 7) is between individuals within capture/miss.

The observations are the means of successful captures for individuals 2, 4, 5, 6 and 7 and the means of unsuccessful captures for individuals 1, 3, 8 and 9.

For the distribution of capture and miss observations across individuals, see Table 1.

\*  $P < 0.025$ .

Table 3. *One-way analysis of variance with treatment (before vs after m. submentalis denervation) as the fixed effect (d.f.=1)*

Dependent variable	Before	After	F-ratio Treatment
	Mean±s.e.	Mean±s.e.	
Duration factor	0.474±0.307	-0.23±0.454	1.705
Minimum mandible angle (degrees)	154.6±3.2	174.6±1.5	10.216*
Tongue factor	0.237±0.331	0.865±0.336	1.398

The observations are the means of all trials for individuals 4-9 before surgery and for individuals 1, 2 and 3 after surgery.

No individuals are used in both treatments.

The residual variance (d.f.=8) is between individual means within before/after surgery.

\*  $P < 0.025$ .

Table 4. *One-way analysis of variance with treatment (before vs after m. genioglossus denervation) as the fixed effect (d.f.=1)*

Dependent variable	Before	After	F-ratio Treatment
	Mean±s.e.	Mean±s.e.	
Duration factor	0.592±0.20	-0.777±0.315	14.584*
Minimum mandible angle (degrees)	149.9±3.1	149.1±2.9	0.025
Tongue factor	0.368±0.305	-1.393±0.083	15.355*

The observations are the means of all trials for individuals 1, 2, 3, 4, 7 and 9 before surgery and for individuals 5, 6 and 8 after surgery.

No individuals are used in both treatments.

The residual variance (d.f.=8) is between individual means within before/after surgery.

\*  $P < 0.025$ .

Tongue protraction factor scores were significantly lower after surgery than before surgery ( $P < 0.025$ , Table 4). Both maximum tongue reach ( $P = 0.0399$ ) and maximum tongue height ( $P = 0.0003$ ) are significantly lower after surgery and contribute significantly to the difference (Table 4, Figs 3C, 4C, 6). Capture success was significantly reduced after (11.1%) denervation of the m. genioglossus (Fisher's exact test,  $P = 0.0114$ ).

### Discussion

The normal feeding behavior of *Discoglossus pictus* is similar to that of the tailed frog *Ascaphus truei*, hypothesized to be the sister group of all other living frogs (Cannatella, 1985). Like *Ascaphus* (Nishikawa and Cannatella, 1991), *Discoglossus* has a tongue of limited protrusibility. In both species, the tongue never protrudes more than 3 mm beyond the tip of the mandibles, and both species lunge forward to catch prey because of their limited tongue protraction abilities (Nishikawa and Cannatella, 1991).

The duration of prey capture, from the onset of mouth opening to the

completion of mouth closing, varies between 112 and 190 ms in *Discoglossus*. Longer durations are associated with the insertion of an intraoral transport cycle into the prey capture cycle before the mouth has fully closed on the prey. This type of variation was also observed in *Ascaphus* (Nishikawa and Cannatella, 1991), but has not been observed in salamanders (Thexton *et al.* 1977; Larsen *et al.* 1989; Findeis and Bemis, 1990; Miller and Larsen, 1990; Reilly and Lauder, 1991).

In *Discoglossus*, successful and unsuccessful capture attempts differ kinematically. When the prey is not contacted successfully, the completion of mouth opening, the completion of tongue protraction and the onset of tongue retraction are postponed while the frog attempts to make successful contact with the prey. Thus, the onset times of those kinematic events that occur after prey contact are delayed and these events require more time to be completed when the prey item is not contacted by the tongue. Similar differences between successful and unsuccessful captures have been observed in *Ascaphus truei* (Nishikawa and Cannatella, 1991). These results suggest that sensory feedback triggers tongue withdrawal during successful prey captures. In the Japanese toad *Bufo japonicus*, a sensory feedback pathway from sensory receptors on the tongue surface to tongue retractor motor neurons, with a latency of 12–20 ms, has been described (Matsushima *et al.* 1988). In *Discoglossus*, the tongue remains at the target for 17–62 ms, which in most cases should be long enough to permit sensory feedback-mediated tongue withdrawal.

In salamanders, there appears to be no difference in feeding kinematics, or in muscle activity, between successful and unsuccessful captures (Reilly and Lauder, 1989; Findeis and Bemis, 1990; Miller and Larsen, 1990; Reilly and Lauder, 1991), and successful captures are much less variable than in frogs (Thexton *et al.* 1977; Lauder and Shaffer, 1985; Larsen *et al.* 1989; Miller and Larsen, 1990). In the salamander *Bolitoglossa occidentalis*, the latency of the tongue withdrawal reflex is at least 12 ms (Thexton *et al.* 1977). Thus, the time between prey contact and the onset of tongue retraction (9–22 ms) is too short for sensory feedback to play a role in tongue withdrawal in these salamanders (Thexton *et al.* 1977).

Denervation of the m. submentalis resulted in a significant decrease in the amount of mandibular bending during feeding. From a resting angle of 180°, the jaw bends down to an angle of 154.6° during normal feeding. After denervation of the m. submentalis, the mandible bends down to an angle of only 174.6°. Thus, the m. submentalis is necessary for mandibular bending. However, neither maximum tongue height nor maximum tongue reach were affected by m. submentalis denervation. Thus, neither mandibular bending nor contraction of the m. submentalis are necessary for tongue protraction in *Discoglossus*. These results contradict the predictions of Gans and Gorniak's (1982a,b) model, in which the m. submentalis acts as a wedge that rises under the stiffened tongue, thereby flipping it over the mandible.

The m. submentalis, a specialization of the m. intermandibularis, is a synapomorphy of all living frogs (Nishikawa and Cannatella, 1991; Duellman and Trueb, 1986). During breathing in frogs, the m. submentalis bends the mandibles

upwards, which pushes the alary cartilages upwards to occlude the external nares as air is forced into the lungs by buccal contraction (de Jongh and Gans, 1969; Gans and Pyles, 1983).

Denervation of the m. genioglossus resulted in a significant decrease in tongue height and tongue reach. Thus, the m. genioglossus is a necessary protractor of the tongue. This indicates that the tongue is not protracted passively by the momentum imparted to it during mouth opening. Without contraction of the m. genioglossus, tongue protraction does not occur. Mandibular bending was not affected by m. genioglossus denervation, and no help from the m. submentalis was necessary for normal tongue protraction.

The m. genioglossus originates broadly in connective tissue near the mandibular symphysis and inserts on the dorsal surface of the tongue. The insertion occupies the entire rostrocaudal and mediolateral surface of the tongue pad. In the model of Gans and Gorniak (1982a,b), the m. genioglossus contracts to stiffen the tongue, but plays no role in tongue protraction. In *Discoglossus*, however, the m. genioglossus does more than stiffen the tongue. When the m. genioglossus contracts, it pulls the tongue pad anteriorly towards the mandibular symphysis. As the fibers of the m. genioglossus shorten, they increase in width and their deformation causes the tongue pad to rise in the floor of the mouth. Thus, contraction of the m. genioglossus alone explains both the change in tongue reach and the change in tongue height during feeding in *Discoglossus*.

Denervation of the m. submentalis and the m. genioglossus both significantly decreased the probability of successful prey capture. The decrease in probability of successful prey capture after m. genioglossus denervation probably results from the failure of tongue protraction. In all but one case, the frogs were unable to transport the prey into the mouth, presumably because the tongue was not protracted. In one case, the prey adhered to the surface of the unprotracted tongue, and was successfully transported into the mouth.

It is unclear why m. submentalis denervation lowered the probability of successful prey capture, because the tongue was protracted normally. Because no treatments were performed in which individuals received anesthesia and surgery but no denervation, general effects of surgery and anesthesia are confounded with the effects of denervation for variables that are similar in both treatments. Thus, decreased capture success in both treatments could be due to a general effect of anesthesia or surgery, especially because the animals were filmed only 30 min after surgery to minimize learning effects. It is important to note, however, that the changes in mandibular bending and tongue protraction that were observed after denervation of the m. submentalis and m. genioglossus, respectively, cannot be artifacts of surgery or anesthesia because they are not the same in both treatments.

A second factor that complicates the interpretation of the results is that successful and unsuccessful attempts at capture were combined in the analyses of the effects of denervation, although they differ kinematically. Thus, kinematic differences before and after surgery could be an effect of the lower probability of successful captures after surgery, rather than an effect of denervation *per se*. In



fact, the duration of kinematic variables is significantly longer after *m. genioglossus* denervation than before denervation. The simplest explanation for this result is (1) that anesthesia decreases the probability of successful capture; and (2) that once an unsuccessful capture attempt begins, it will be characterized by longer durations of kinematic variables owing to the absence of sensory feedback elicitation of the tongue withdrawal reflex.

It is important to note, however, that neither the decrease in tongue protraction after *m. genioglossus* denervation nor the decrease in mandibular bending after *m. submentalis* denervation can be explained as a consequence of the greater number of unsuccessful attempts at capture after surgery. Neither tongue protraction nor mandibular bending differed significantly between successful and unsuccessful attempts at capture before surgery (Table 1).

The results of these experiments show that kinematic analysis of feeding behavior before and after denervation of selected muscles is a technique that yields interpretable data that may be used to test hypotheses of muscle function. Surgical denervation of the *m. submentalis* affected mandibular bending but not tongue protraction, and denervation of the *m. genioglossus* affected tongue height and tongue reach, but not mandibular bending. None of the results suggests that transection of sensory fibers disrupted feedback, or that learned adjustment of feeding after surgery appears to complicate the results. The only non-specific changes that occurred in feeding behavior after surgery were apparently due to the decreased probability of successful capture.

These denervation experiments show that the *m. submentalis* bends the mandibles but is neither necessary nor sufficient for tongue protraction, while the *m. genioglossus*, in addition to stiffening the tongue, is a necessary protractor of the tongue in *Discoglossus pictus*. While these results contradict the predictions of Gans and Gorniak's (1982*a,b*) model of tongue protraction, they do not disprove it. The model was developed for *Bufo marinus*, a neobatrachian frog with a highly projectile tongue. If the rod and wedge model is correct for *Bufo*, then *Discoglossus* must have a different tongue protraction mechanism. Denervation of the *mm. submentalis* and *genioglossus* in *Bufo marinus* will be necessary to test this hypothesis.

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