

THE MECHANISM OF TONGUE PROJECTION IN CHAMELEONS

II. ROLE OF SHAPE CHANGE IN A MUSCULAR HYDROSTAT

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Summary

In this paper we investigate the interaction between the accelerator muscle (the muscle that powers tongue projection) and the entoglossal process (the tongue's skeletal support) that occurs during tongue projection in chamaeleonid lizards. Previous work has shown that there is a delay of about 185 ms between the onset of accelerator muscle activity and the onset of tongue projection. In conjunction with anatomical observations, *in vitro* preparations of the accelerator muscle mounted on isolated entoglossal and surrogate processes were stimulated tetanically, and the resulting movements were recorded on video at 200 fields s⁻¹. Three results indicate that morphological features of the entoglossus and the accelerator muscle delay the onset of tongue projection following the onset of accelerator contraction: (1) the entoglossus is parallel-sided along the posterior 90 % of its shaft, only tapering at the very tip, (2) the sphincter-like portion of the accelerator muscle, which effects tongue projection, makes up the posterior 63 % of the muscle and does not contact the tapered region of the entoglossus at rest, and (3) accelerator muscles mounted on the entoglossus undergo longitudinal extension and lateral constriction for 83 ms following the onset of electrical stimulation, before projecting off the entoglossus. It is proposed that, during elongation of the accelerator muscle, the sphincter-like region ultimately comes into contact with the tapered region of the entoglossus, causing the onset of projection. This conclusion is supported by the observation that the time between the onset of stimulation and the onset of projection was longer in preparations with surrogate entoglossal processes that had no tapered tip and shorter with surrogate processes that had a tapered tip about four times as long as the natural entoglossus.

Tetanically stimulated accelerator muscles reached 90 % of peak force 110 ms after the onset of stimulation, indicating that the 185 ms delay between the onset of accelerator activity and the onset of projection seen *in vivo* allows the accelerator to achieve peak force prior to the onset of projection. Thus, the delay

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in projection may be crucial in maximizing the acceleration and velocity achieved by the projected chameleon tongue.

Introduction

The amniote tongue is characterized by a large, complexly arranged muscular portion invested with skeletal elements of the hyobranchium. Tongue movements can be caused either by muscles that attach to the hyobranchium or by the actions of intrinsic tongue muscles. Unlike most vertebrate skeletal muscles, many intrinsic tongue muscles do not have skeletal attachments, yet these muscular hydrostats are capable of producing extensive and intricate tongue movements (e.g. Smith, 1984, 1986, 1988). Kier and Smith (1985) pointed out that the constant-volume nature of muscular hydrostats is their most important mechanical feature. Because they maintain a constant volume, changes in any dimension will result in compensatory changes in other dimensions. The mechanisms of elongation, bending and torsion in muscular hydrostats all depend on constancy of volume to effect shape changes in the absence of stiff skeletal attachments (Kier and Smith, 1985). In this paper we explore the role of muscular shape change in the tongue projection mechanism of chamaeleonid lizards.

The chameleon tongue is invested with an elongate entoglossal process that enters a lumen in the centre of the tongue and runs anteriorly to the tongue's tip (Gnanamuthu, 1930; Zoond, 1933; Gans, 1967; Bell, 1989; Wainwright and Bennett, 1992, Figs 1–3). Contraction of the sphincter-like accelerator muscle against the entoglossal process causes the tongue to be projected off the process (Zoond, 1933). Electromyographic recordings, synchronized with high-speed video recordings in *Chamaeleo jacksonii* (Wainwright and Bennett, 1992), have shown that the accelerator muscle begins intense, continuous electrical activity about 185 ms prior to the onset of tongue projection. In this paper we investigate the interaction between the accelerator muscle and the entoglossal process that may cause this time lag between the onset of muscular contraction and tongue projection. *In vitro* preparations of the accelerator muscle mounted on the entoglossus and three surrogate processes were stimulated electrically and the resulting accelerator movements recorded on high-speed video. The purpose of the three surrogate preparations was to explore the function of the shape of the entoglossus in its interaction with the accelerator muscle. The results indicate (1) that key morphological features of the entoglossus and accelerator muscle prevent the accelerator from initiating projection immediately following the onset of contraction, and (2) that shape changes of the accelerator muscle that occur during contraction alter its relationship with the entoglossus, ultimately permitting projection.

Materials and methods

Anatomy

Observations and experimental work were performed with 16 *Chamaeleo*

jacksonii Boulenger ranging between 86 and 117 mm (mean=104.7 mm) snout-vent length (SVL). All individuals were collected during March 1989 in Nairobi, Kenya, under permit no. OP.13/001/18c94/19 to A.F.B. Animals were transported to the University of California, Irvine, where they were housed under a 12h:12h L:D cycle and provided with water and live crickets regularly. Anatomical observations and measurements were made on the tongue and hyobranchium of five individuals (98.3–111.2 mm SVL) within 30 min of being killed with an overdose of Halothane gas anaesthesia (all animals used in this study were killed in this way). Fresh specimens were used to avoid the possible morphological distortions caused by tissue fixation. The hyobranchium and tongue were dissected from each individual and the length and width of the accelerator muscle were measured as it rested on the elongate anterior portion of the hyobranchium, the entoglossal process. The tongue was then separated from the skeletal elements and a series of measurements was made on the length and diameter of the entoglossus. Diameter was measured every 3 mm along the length of the entoglossus and every 1 or 2 mm near the anterior tip. All measurements were made with an accuracy of ± 0.025 mm under a dissecting microscope equipped with an ocular micrometer.

Accelerator with entoglossal process

A series of observations was made on *in vitro* preparations of the accelerator muscle to understand better the dynamic relationship between this muscle and the entoglossal process. The first preparation was designed to elucidate the timing of accelerator muscle movements during contraction while mounted on the entoglossal process. Because the tongue is held mostly within the margin of the gape during the early stages of tongue projection in live chameleons, it is not possible *in vivo* to monitor detailed movements of all parts of the tongue during the critical period when it is accelerated forward towards the prey. The hyobranchium and intrinsic tongue musculature were removed from three killed individuals (97.4, 102.2 and 106.3 mm SVL) and placed in a dish of saline (all saline used in this study contained 145 mmol l^{-1} NaCl, 4 mmol l^{-1} KCl, 20 mmol l^{-1} Hepes, 2 mmol l^{-1} CaCl₂ and 2 g l^{-1} glucose adjusted to pH 7.6 at 23°C). The accelerator muscle is arranged as a complex sphincter muscle around a central lumen which ends in a blind sac (see Fig. 2 in Wainwright and Bennett, 1992). The nerves that follow the hyoglossi muscles and innervate the accelerator muscle were severed and separated from surrounding tissue so that 5 cm sections of each nerve were exposed before their entry into the posterior region of the accelerator muscle. This dissection required that the hyoglossi be transected near their attachment to the hyobranchium, but they were otherwise left intact throughout the experiment. The hyobranchium, with tongue in place, was then mounted in a clip and oriented 60° above the horizontal to prevent the tongue from sliding off the entoglossus. The exposed nerve sections were draped across silver wire electrodes and tetanic stimuli of 400 ms duration were delivered to the accelerator muscle at 40 Hz by a Grass SD5 stimulator. The muscles and nerves were frequently flushed with fresh

saline but the observations took place in air, not submerged in saline. Following each stimulation bout the tongue was remounted on the entoglossus manually.

Movements of the stimulated muscles were taped with a NACHSV 400 video system recording $200 \text{ fields s}^{-1}$ using one strobe for lighting. To synchronize the electrical stimulation with the video record the stimulus pulse sent to the muscle was split and recorded as an analogue trace on the video screen. The video record of three contraction events from two muscle preparations and two contractions from a third preparation were analyzed field-by-field with the aid of a computer-based analysis system (total $N=8$). Beginning several frames prior to the onset of the stimulus, the diameter of the accelerator muscle and the positions of the posterior margin and the anterior tip of the accelerator muscle were measured along a line continuous with the central axis of the entoglossal process.

Accelerator with surrogate entoglossus

A series of three accelerator muscle preparations was used to determine the role of entoglossal shape in the interaction between it and the accelerator muscle. In the first, the entoglossal process was removed from the accelerator lumen of three individuals (89.1, 93.3 and 93.2 mm SVL) and replaced with a $20 \mu\text{l}$ micropipette. The micropipettes had parallel glass walls and smooth, squared ends with no taper. The mean entoglossus shaft diameter of the three chameleon specimens was 1.27 mm, compared to 1.35 mm outer diameter of the micropipettes. The preparation was mounted in a clip and the accelerator nerves were exposed and stimulated under the same conditions described in the section above. Muscle contractions synchronized with the electrical stimulus were recorded on video at $200 \text{ fields s}^{-1}$. Sequences from three tetanic contractions for one preparation and two contractions from each of two other muscles were analyzed (total $N=7$). For each sequence, every video field was analyzed beginning several fields prior to the onset of the stimulus and continuing until after the accelerator had lost contact with the micropipette. From each frame, the positions of the posterior margin of the accelerator muscle and the anterior position of the accelerator were measured along a line continuous with the tip of the entoglossus.

In a second preparation, the entoglossus from two of the accelerator muscles was replaced with a micropipette that had been hand-drawn into a 9.3 mm taper that reduced from 1.35 mm outer diameter to 0.19 mm diameter at its tip, resulting in an average angle of 7.1° along the 9.3 mm taper. In these preparations, the anterior part of the circular region of the accelerator muscle surrounded the tapered micropipette, and the posterior part of the circular region surrounded the parallel-sided base of the micropipette. The accelerator was stimulated through the exposed nerves following the same protocol used in the other preparations, and the resulting movements were synchronized and recorded on video at $200 \text{ fields s}^{-1}$. Movements of the posterior and anterior margins of the accelerator muscle were measured in consecutive video fields beginning several fields prior to the onset of the electrical stimulus until about 150 ms after stimulation (total $N=5$).

To compare the effect of the shape of the process tip on the timing of tongue projection, a pair of nested analyses of variance (ANOVAs) was run on the time between the onset of the stimulus and the onset of projection. In one analysis the contrast was made between the entoglossus preparation and the square-ended micropipette. In the other analysis the contrast was made between the entoglossus preparation and the tapered micropipette. Both ANOVAs were two-level nested designs with individual muscle preparation nested within process type. The *F*-ratios used to test the process-type factor were constructed with the mean squares of the process-type effect in the numerator and the mean square for individuals nested within process-type in the denominator (Sokal and Rohlf, 1981).

Following the observations described above, a second set of observations was made with three muscles. The micropipette was forced through the membrane at the anterior tip of the accelerator lumen and moved forward until 6–7 cm of the glass tube projected anteriorly beyond the muscle and an equal length projected posteriorly from the entry to the lumen. The preparation was mounted horizontally and the muscle was tetanically stimulated through the accelerator nerves. The resulting contraction was synchronized with the electrical stimulus and recorded on video at $200 \text{ fields s}^{-1}$. Movements of the posterior and anterior margins of the accelerator muscle were measured in consecutive video fields from several fields prior to stimulation until 150 ms after the onset of stimulation (total $N=5$).

Contractile measurements

The time course of mechanical activity was documented during contraction in the accelerator muscle. We chose to measure pressure within the fluid-filled accelerator lumen rather than use the more conventional technique of directly measuring tension of the muscle or bundles of its fibres. Pressure was chosen because we felt it better reflected the novel structure and function of this muscle as it interacts with the entoglossal process and because the measurements could be made on the intact muscle.

Immediately after an animal had been killed, the accelerator muscle and tongue pad were separated from the bodies of four animals by severing the hyoglossi muscles at their attachment to the posterior margin of the accelerator muscle. The muscle was then placed directly into a dish filled with a fresh solution of saline.

Each accelerator muscle was prepared by first glueing (cyanoacrylate adhesive) a 3.0 cm section of stiff plastic tubing (0.88 mm i.d.) to the opening of the lumen at the posterior end of the muscle. The end of the tube was flanged to provide a broad area of attachment between the plastic tube and the thick fascia that surrounds the muscle. A section of glass tubing (0.53 mm o.d.) was then inserted into the end of the lumen through the plastic cannula so that it emerged approximately 2 mm into the cannula. The glass tubing provided a stiff central rod, similar to the entoglossal process, which prevented the accelerator from buckling during contraction. The lumen of the accelerator and the plastic tube were then filled with saline solution through a hypodermic syringe inserted into the section of glass tubing. A Millar

PC-350 catheter-tipped pressure transducer was threaded into the cannula so that it rested about 3 mm from the opening of the lumen. The pressure transducer had a frequency response greater than 1000 Hz. The junction between the pressure sensor and the tubing was then sealed with a silicone adhesive so that the pressure sensor was in a closed, fluid-filled system. The accelerator was then placed in a recirculating bath of temperature-controlled saline and electrical stimulations were administered by a Grass SD5 stimulator through adjacent 1 cm×3 cm platinum plate electrodes. Maximal tetanic stimulation was obtained at 23°C with 800 ms pulse trains between 35–45 Hz. The pressure record from the Millar transducer and the electrical stimulus were simultaneously recorded on two channels of a Hewlett-Packard 4086 multichannel FM instrumentation recorder. Data were later played back for analysis at one-eighth recorded speed on a Gould 3600 chart recorder.

Measurements were made on four or five tetanic pressure traces for each of the four muscle preparations (total $N=18$). For each tetanic trace four variables were measured: (1) the time from the onset of pressure development to 90 % of peak pressure, (2) the time from the onset of the electrical stimulus to 90 % of peak pressure, (3) the time from the last stimulus pulse until the muscle relaxed to 90 % of peak pressure, and (4) the percentage of peak pressure that the muscle retained 30 ms after the last stimulus pulse. Temporal variables were measured to the nearest 2.5 ms.

Results

Anatomy

Detailed descriptions of the morphology of the cranial region of *Chamaeleo* have been presented elsewhere (Mivart, 1870; Gnanamuthu, 1930, 1937; Rieppel, 1981; Tanner and Avery, 1982; Schwenk and Bell, 1988; Bell, 1989; Wainwright *et al.* 1991; Wainwright and Bennett, 1992). Here we focus on anatomical details of the accelerator muscle and entoglossal process that have not previously been noted or are crucial to our interpretation of the tongue projection mechanism.

Among the five individuals examined [SVL (mm)=98.3, 103.2, 106.8, 108.1, 111.2], the entoglossal process was about 25 % of SVL (Table 1). In its relaxed state, the accelerator muscle covers the anterior 61 % of the entoglossus (Table 1 and Fig. 1). The accelerator muscle itself is divided into two distinct regions. The posterior region forms a complete ring around the lumen and makes up 61 % of the length of the accelerator muscle (Table 1). The remaining anterior section does not form a continuous ring around the lumen but exists as an extension of the muscle ventral to the lumen that continues to the anterior tip of the tongue (Fig. 1 and Wainwright and Bennett, 1992, Fig. 2).

All five entoglossal processes measured in this study were parallel-sided along the posterior 91 % of their length (Table 1 and Fig. 1). Thus, in none of the five specimens did the diameter of the process change significantly along the posterior 90 % of its length. Only the anterior 9 % of the entoglossus exhibited a taper,

Table 1. Morphometrics of the entoglossal process and accelerator muscle from a sample of five *Chamaeleo jacksonii*

| Structure | Mean length (mm) | Percentage of total structure length |
|----------------------|------------------|--------------------------------------|
| Snout-vent length | 105.5±4.95 | |
| Entoglossal process | | |
| Total length | 26.2±0.8 | |
| Parallel-sided shaft | 23.8±0.7 | 91.1 |
| Tapered region | 2.35±0.2 | 8.97 |
| Accelerator muscle | | |
| Total length | 16.1±3.8 | |
| Circular region | 9.8±2.1 | 60.9 |
| Non-circular region | 6.3±1.5 | 39.1 |

See Fig. 1 for matching diagram.

Values for length are mean±s.e.

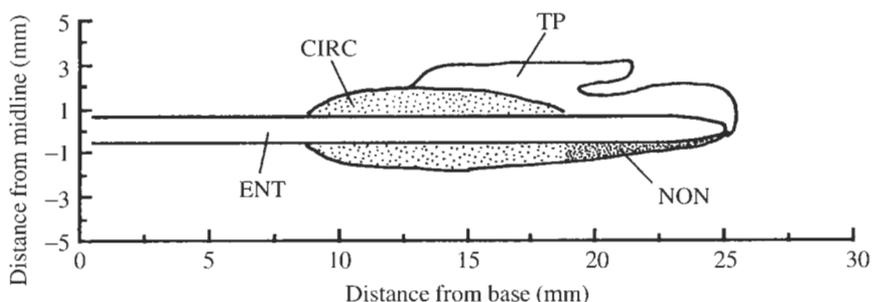


Fig. 1. Diagram of the tongue tip and entoglossal process in *Chamaeleo jacksonii*. The entoglossus is parallel-sided along the posterior 90% of its length, tapering only at the tip. The portion of the accelerator muscle that surrounds the entoglossus (lightly stippled region) forms the posterior 63% of the accelerator muscle and therefore does not contact the tapered region of the entoglossus while at rest. See Table 1 for quantified morphometrics. CIRC, circular region of the accelerator muscle; NON, anterior, non-circular region of the accelerator muscle (heavily stippled region); ENT, entoglossus; TP, tongue pad.

which narrowed at about a 20° angle (mean=19.4°; s.e.=1.9°), reducing to about 40% of the shaft diameter at its tip, where the process terminates in a rounded end (Fig. 1 and Table 1).

In a relaxed state the circular portion of the accelerator muscle surrounds a region of the entoglossus posterior to the tapered tip of the process (Fig. 1 and Table 1). In one 98.3 mm SVL individual, the entoglossal process was 24.5 mm long and its tapered tip formed the anterior 2.5 mm of the process. The anterior, non-circular portion of the accelerator muscle was 5.9 mm long, fully overlapping the tapered tip of the entoglossus and reaching another 3.4 mm posterior to the

tapered section. Thus, the 9.1 mm of circular accelerator muscle surrounded the parallel-sided shaft of the entoglossus, reaching anteriorly to within 3.4 mm behind the tapered region (Fig. 1).

Accelerator with entoglossus

All stimulated accelerator muscles contracted and projected themselves off the tip of the entoglossus. However, projection did not commence immediately after the onset of stimulation and velocities never achieved more than about 0.8 m s^{-1} , compared to peak tongue velocities of about 4 m s^{-1} , which we have observed in naturally feeding individuals of this species. No movement was observed in the muscle until about 18 ms following the onset of the stimulus train (mean=18.4 ms, s.e.=4.1 ms; Fig. 2). At this time, the anterior margin of the accelerator began to extend forward, while the posterior margin of the muscle either did not move or extended posteriorly very slightly (Fig. 2). This pattern continued for about 65 ms (mean=64.8 ms, s.e.=7.8 ms), during which time the length of the accelerator muscle increased by an average of 47% (mean=47.1%, s.e.=8.8%) and the width of the muscle decreased by 15% (mean=14.5%, s.e.=3.1%). During this period of lateral constriction and elongation of the accelerator, the volume of the muscle, as calculated from the width and length measures, did not change significantly (Fig. 2). Beginning 83 ms (mean=83.4 ms, s.e.=10.9 ms) after the onset of the stimulus, the anterior and posterior margin of the muscle abruptly began to accelerate and moved forward rapidly for about 20 ms until the tongue slid off the end of the entoglossus (Fig. 2).

Accelerator with surrogate entoglossus

When the entoglossus was replaced with a square-ended micropipette, the pattern of movement of the stimulated accelerator muscle was similar to the pattern observed with the entoglossus. The muscle contracted, became more elongate and rapidly slipped off the end of the micropipette (Fig. 3A). Among the seven contractile sequences recorded on video, the first movements of the accelerator began about 20 ms (mean=20.4 ms, s.e.=5.7 ms) after the onset of the stimulus train. Extension of the anterior margin of the accelerator coupled with slight posterior extension of the posterior margin occurred over a 94 ms period

Fig. 2. Kinematic data taken from video records of an accelerator muscle, mounted on an isolated entoglossus during tetanic stimulation through the exposed nerves. In this trial the muscle began to change shape about 15 ms following the onset of the stimulus, elongating and constricting laterally until 75 ms after stimulus onset. The period of muscle elongation is indicated by the shaded bar. The muscle elongated mostly by anterior extension. At 75 ms, the entire muscle suddenly began to move forward, projecting itself off the end of the entoglossus. In this figure and Fig. 3 the onset of tongue projection was defined as the video field immediately prior to the time when both the anterior and posterior margins of the accelerator muscle move anteriorly at least 1.0 mm. During the period of muscle elongation between the onset of stimulation and the onset of projection, the volume of the muscle, calculated from the measured length and width, remains unchanged.

(mean=94.4 ms, s.e.=9.8 ms). During this time the accelerator increased in total length by an average of 44.2% (s.e.=5.7%), while the muscle constricted by an average of 12.6% (s.e.=2.1%). Beginning 114 ms (mean 114.3 ms, s.e.=11.1 ms)

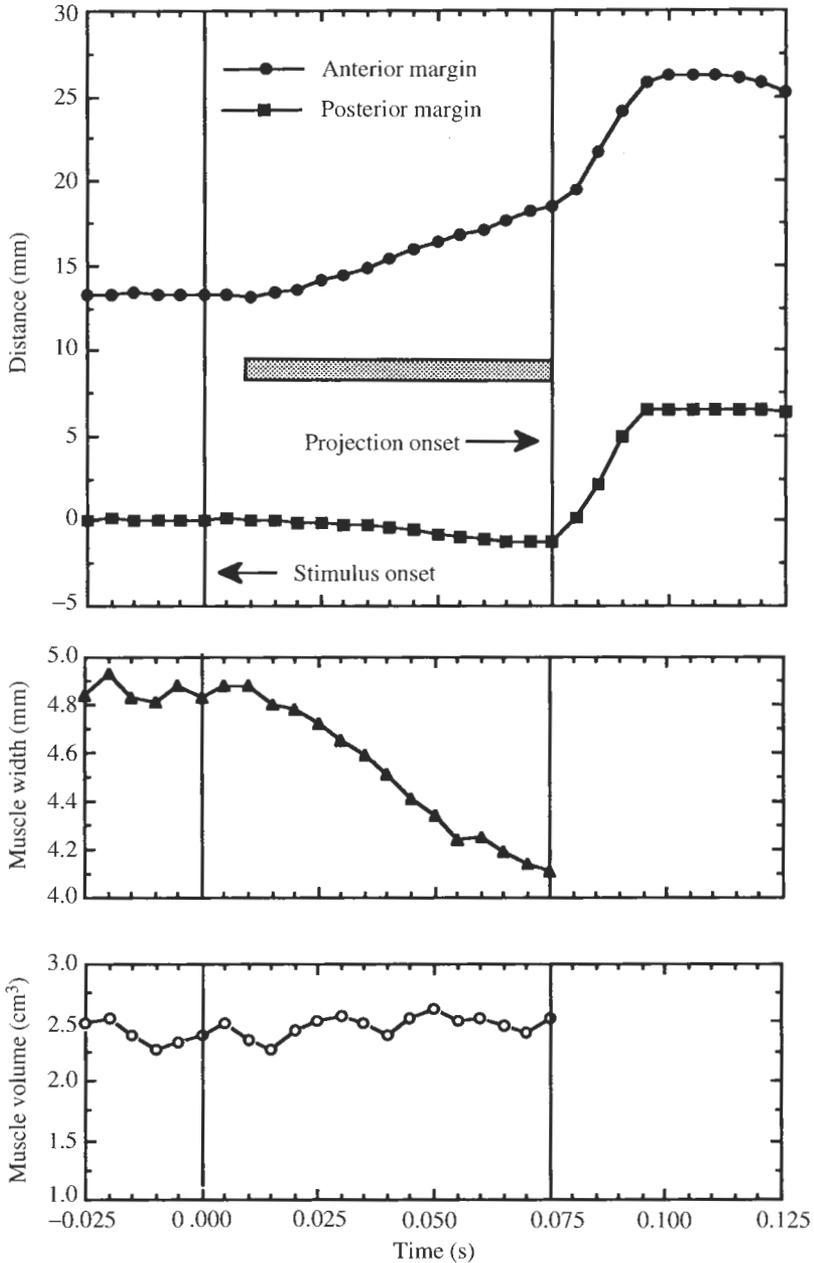


Fig. 2

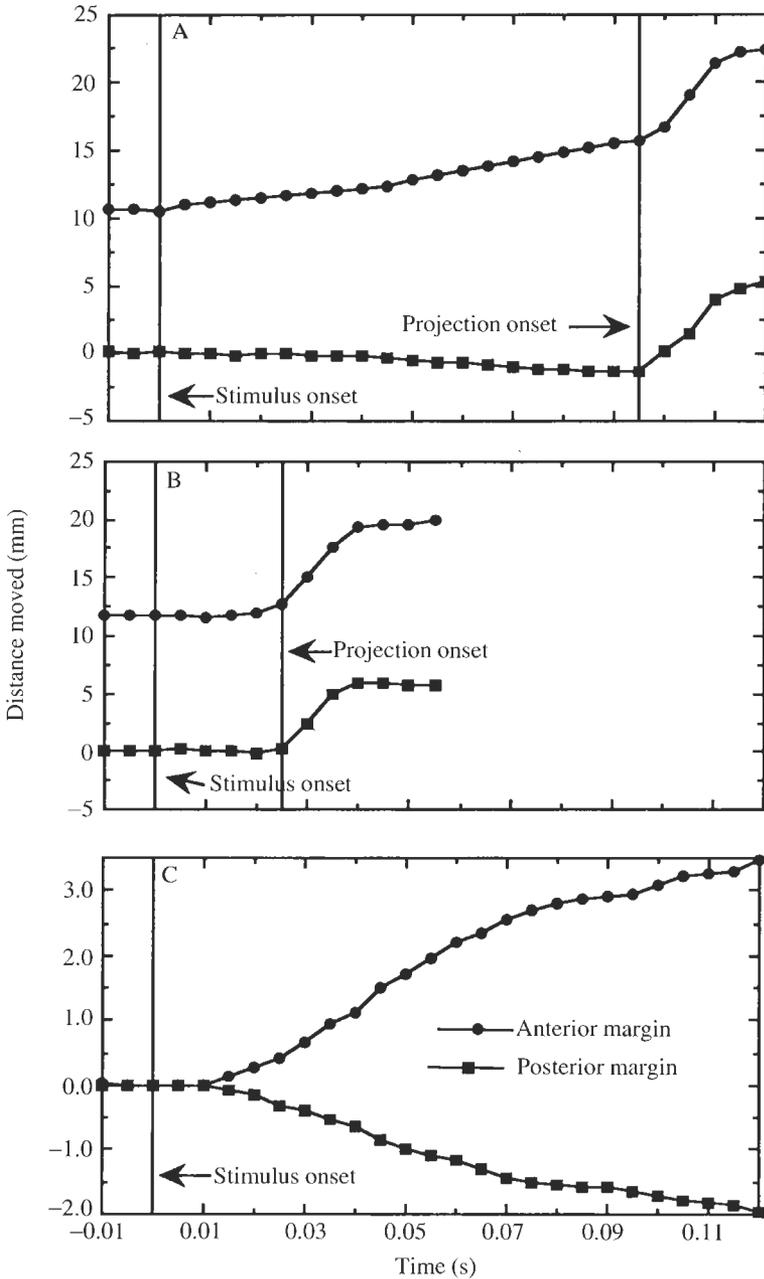


Fig. 3

after the onset of the stimulus train, both the anterior and posterior margins of the accelerator rapidly moved forward for 20 ms until the muscle slid off the end of the micropipette (Fig. 3A). A nested analysis of variance (Table 2) contrasting this

Fig. 3. Kinematic data from video records of three accelerator muscle preparations. (A) In this preparation the entoglossus was replaced with a square-ended micropipette. Movements of the accelerator were similar to those seen when the entoglossus was used (Fig. 2), except that the period of muscle elongation between stimulus onset and the onset of tongue projection was significantly longer (Table 2). (B) The entoglossus was replaced with a micropipette with an extended 9.3 mm taper. In this preparation the circular region of the accelerator muscle was in contact with the tapered section of the surrogate entoglossus from the onset of the trial. Accelerator muscle kinematics were similar to those seen with an intact entoglossus, except that the time between stimulus onset and the onset of projection was shorter (Table 2). (C) In this preparation the square-ended micropipette was forced through the anterior end of the accelerator muscle, so that the accelerator was not in contact with the tip of the surrogate entoglossus at any point during the trial. The accelerator extended anteriorly and posteriorly but no net translation along the micropipette was observed.

Table 2. Results of two nested analyses of variance contrasting the time between the onset of electrical stimulation and the onset of tongue projection in three accelerator muscle preparations

| | <i>F</i> | d.f. | <i>P</i> |
|--|----------|------|----------|
| Entoglossus vs square-ended micropipette | | | |
| Process type | 12.6 | 1, 4 | 0.022 |
| Individuals | 0.60 | 4, 9 | 0.671 |
| Entoglossus vs tapered micropipette | | | |
| Process type | 24.1 | 1, 3 | 0.016 |
| Individuals | 0.36 | 3, 8 | 0.782 |

In each analysis individual preparations were nested within type of experimental entoglossal process (see text).

preparation with the results obtained with the intact entoglossus found a significant effect of process type, with the square-ended preparation producing a longer delay between stimulus onset and projection onset (means=83.4 ms, intact, and 114.3 ms, square-ended preparation).

Muscles mounted on a tapered micropipette slipped off the end of the glass tube when stimulated. However, these preparations did not show the prolonged period of muscle extension that preceded projection in the preparations with an intact entoglossus or a square-ended micropipette (Fig. 3B). Muscle movement commenced an average of 18.1 ms after the onset of the stimulus train (s.e.=9.1 ms). Initially, the anterior margin of the muscle began to extend rostrally and the posterior margin extended caudally as in the other preparations. However, only 12.3 ms (s.e.=6.3 ms) after this movement started, the posterior margin of the muscle abruptly reversed direction and the entire muscle began to slide off the end of the micropipette. The period between stimulus onset and projection onset

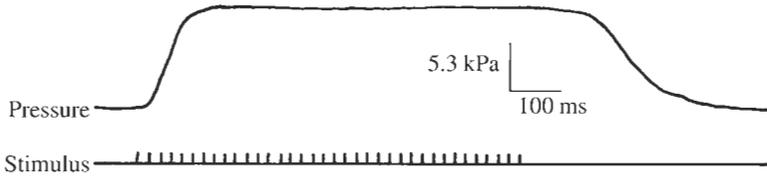


Fig. 4. Pressure record made inside the lumen of the accelerator muscle during tetanic stimulation. Note that peak pressure is not achieved for more than 100 ms following the stimulus onset and the muscle retains near maximal pressure for at least 50 ms following the termination of the stimulus train. See text for details of preparation and contractile variables.

was significantly briefer than that observed in the intact preparation (Table 2; means=83.4 ms, intact, and 30.3 ms, tapered micropipette).

Accelerator muscles that were mounted on the micropipette with several centimetres emerging anteriorly and posteriorly exhibited a distinctly different movement pattern from any of the preparations described above (Fig. 3C). Muscle movement began about 15 ms following the onset of the stimulus (mean=14.8 ms, s.e.=8.9 ms). At this time the anterior margin of the muscle extended forward along the micropipette while the posterior margin of the muscle extended posteriorly at a slightly slower rate (Fig. 3C). During this time, the muscle constricted. The overall length of the muscle increased by about 61 % (mean=61.3 %, s.e.=12.3 %) and muscle shape stopped changing about 260 ms after the onset of the stimulus (mean=259.5 ms, s.e.=24.8 ms). Thus, no net anteriorly directed movement of the accelerator muscle occurred during contraction and the muscle did not slide off the end of the micropipette.

Accelerator contractile kinetics

Tetanic pressure traces (Fig. 4) exhibited the classical shape seen in more conventional tetanic tension measurements in skeletal muscle from ectotherms (Akster *et al.* 1985) and other chameleon muscles (Abu-Ghalyun *et al.* 1988). Pressure began to increase an average of 13.5 ms following the first stimulus pulse in the train and rose rapidly until it plateaued at its maximum value (Fig. 4). Average peak pressures of 11.3 kPa (s.e.=0.9 kPa) were reached. Time from the onset of pressure change to 90 % of peak pressure averaged 110.4 ms (s.e.=9.8 ms) and time from stimulus onset to 90 % peak pressure was 123.9 ms (s.e.=8.3 ms). The time required for the accelerator muscle to relax to 90 % of peak pressure following the offset of the stimulus was 154 ms (s.e.=12.8 ms). In all 18 tetanic sequences analyzed, the muscle maintained 100 % of peak pressure for 30 ms after the offset of the stimulus train. Pressure traces appeared to vary more among preparations than among stimulus bouts within each muscle.

Discussion

The function of the accelerator muscle during tongue projection appears to

depend on shape changes that occur during its contraction. The accelerator, a sphincter muscle, exerts forces against the entoglossal process that result in ballistic projection of the tongue (Zoond, 1933). Electromyographic data from free-feeding animals indicate that the accelerator muscle begins contraction an average of 187 ms prior to the onset of tongue projection and continues activity until about 11 ms prior to tongue projection (see Table 1 in Wainwright and Bennett, 1992). This result creates two questions. Why is tongue projection delayed by an average of 187 ms after the onset of accelerator contraction and what are the mechanical consequences of this delay? Based on the results presented in this paper, we present a scenario for the interaction between the accelerator muscle and the entoglossus during the tongue projection sequence that accounts for the observed timing of accelerator muscle activity. Detailed explanations follow the overall interpretation.

One of the central findings of this study is that morphological features of the entoglossus and the accelerator muscle prevent the contraction of the accelerator muscle from immediately causing projection. The entoglossus is parallel-sided along most of its length, tapering only along the anterior 9% (Fig. 1). During contraction, the accelerator muscle exerts compressive forces against the long axis of the entoglossus, but the parallel sides of the entoglossus shaft yield no anteriorly directed force vector. Only the tip of the entoglossus has the tapered shape that will permit the accelerator to generate projectile forces. Furthermore, the ability to exert compressive forces will be restricted to the portion of the accelerator muscle that completely surrounds the entoglossus (hereafter referred to as the 'circular' region). It appears that a critical aspect of the interaction between the accelerator muscle and the entoglossus is that, prior to the onset of the projection sequence, the circular region of the accelerator muscle is not in contact with the tapered tip of the entoglossus (Fig. 1). Therefore, the accelerator acts on the parallel-sided entoglossus shaft at the onset of its contraction. As the fibres of the accelerator muscle shorten, the diameter of the muscle decreases (Fig. 2). Since muscle volume remains constant, the accelerator muscle extends longitudinally, accommodating the reduction in cross-sectional area. The accelerator muscle does not begin to exert forces with an anterior vector until it has extended onto the tapered region of the entoglossus. Thus, there is a delay between the onset of accelerator contraction and the onset of tongue projection during which the accelerator muscle extends longitudinally until it is exerting compressive forces against the tapered region of the entoglossus. Only then can projection commence.

Shape changes of the accelerator, monitored while the muscle was mounted in isolation on the entoglossus, indicate that the muscle begins to constrict laterally and to elongate within 19 ms of the onset of electrical stimulation (Fig. 2). Elongation occurred mostly in an anterior direction rather than symmetrically (Fig. 2). Following about 87 ms of elongation (106 ms following the onset of electrical stimulus), the entire muscle abruptly began to move forward, sliding off the end of the entoglossus. The preparation portrayed in Fig. 2 included an

entoglossus 27 mm long with a 2.8 mm tapered tip and an accelerator muscle divided into an anterior 6.4 mm non-circular region and a posterior 10.8 mm circular region. Thus, at rest the circular region reached anteriorly to within approximately 3.6 mm of the tapered region. Extension of the accelerator following stimulation moved the anterior margin of the tongue pad 5.0 mm from its starting point (Fig. 2). It was not possible to isolate the extension of only the circular region of the accelerator in this preparation, but given the fact that the circular region makes up about 63% of the length of the entire muscle, and assuming a constant degree of elongation along the length of the muscle, a 5.0 mm increase in overall muscle length would result in an increase in the length of the circular region of 3.15 mm. This is approximately the distance between the original anterior extension of the circular region and the posterior margin of the tapered region (3.6 mm).

The ability of the accelerator muscle to force itself off the entoglossus appears not to require the short tapered region of the process (Fig. 3B). Following replacement of the entoglossus with a square-ended glass micropipette, the accelerator muscles exhibited a pattern of movement that was similar to that of the preparations that included the real entoglossus (compare Figs 2 and 3), except that the period of muscle elongation was longer in the preparations with the micropipette. The ends of the glass tubes were squared and polished so that virtually no tapered region was present.

In contrast, accelerator muscles mounted on a tapered micropipette showed a much briefer period of elongation prior to projection. Muscles mounted on the tapered micropipette began their projectile motion 12.3 ms after the contraction began, compared to 65 ms when mounted on the entoglossus and 94 ms when mounted on the square-ended micropipette. Our interpretation of this much shorter period of elongation is that, because the tapered tip of the micropipette was 9.3 mm long, the circular region of the accelerator muscles in these preparations was contracting against a tapered structure from the onset of their activity and, therefore, began sliding anteriorly almost immediately.

The accelerator muscles that were mounted on a micropipette in such a way that they were not in contact with the end of the micropipette exhibited no net anteriorly directed movement. Because the muscles in these preparations were in contact with a parallel-sided structure throughout contraction, their interaction with the glass tube would not be expected to result in directional muscle movement. The movements observed during contraction of these preparations were a reduction in muscle diameter, some posterior extension, and somewhat greater anterior elongation (Fig. 3C). It is noteworthy that only during these contractions did the posterior margin of the muscle show about the same degree of movement as the anterior margin of the muscle. When mounted on the end of a micropipette, or on an entoglossus, the anterior margin of the accelerator muscle showed distinctly greater movement than the posterior margin (compare Figs 2 and 3A with Fig. 3C). One explanation for this tendency is that in those preparations that permitted initial contact of the anterior region of the tongue with

the tip of the entoglossus or micropipette, the muscle and tongue pad may have readily moved anteriorly to collapse on the vacated lumen. Observations on stimulated accelerator muscles, in the complete absence of an entoglossus, suggest that the muscle does collapse the lumen as it contracts.

Contracting accelerator muscle preparations did eventually stop elongating (Fig. 3C), a fact that may play an important role in the function of this region of the tongue after the tongue is launched towards the prey. Electromyographic recording from the accelerator muscle indicates that the muscle is active for an average of 566 ms following the onset of projection (Wainwright and Bennett, 1992). During this time the tongue makes contact with the prey and is retracted into the mouth. During most of this time the accelerator muscle may be fully elongated and acting as a stiff support against which the intrinsic muscles of the tongue pad may operate. One mechanism that may ultimately limit accelerator muscle elongation resides in the skin surrounding the muscle. The accelerator muscle is enclosed in a connective tissue sheath formed of fibres oriented in a cross-helical array (P. C. Wainwright and A. F. Bennett, unpublished observations). As the accelerator muscle elongates and constricts laterally during contraction, the angle formed by the fibres in the skin and the long axis of the muscle will be expected to decrease (Clark and Cowey, 1958). As the angle decreases below 54° , tension will increase in the fibres until the force created by the contracting muscle is offset by the longitudinal tension in the fibres, ultimately restricting or preventing further elongation of the muscle.

Mechanical consequences of delayed projection

The acceleration and velocity achieved by a projectile are a function of the mass of the object and the force that powers its launch (Alexander, 1971). Maximum tongue acceleration of 486 ms^{-2} and a peak velocity of 5.8 ms^{-1} have been reported for unrestrained individuals of *Chamaeleo oustaleti* feeding at 30°C body temperature (Wainwright *et al.* 1991). Bell (1990) reported average peak tongue velocities of 4.25 m s^{-1} in *C. zeylanicus* and *C. pardalis*. For a projectile system to maximize acceleration and velocity, it is necessary to maximize the force that the system exerts during the launching phase. Morphological features of the accelerator and entoglossus cause tongue projection to be delayed following the onset of muscle contraction. This delay of about 187 ms (Wainwright and Bennett, 1992) has an important mechanical consequence for accelerator muscle function. When stimulated tetanically at 23°C , the accelerator required about 110 ms to reach 90 % of peak pressure (Fig. 4). A delay in projection onset of 187 ms following the onset of muscle contraction permits the accelerator time to develop peak force. Accelerator preparations mounted on a long-tapered micropipette began projection 12 ms after the onset of muscle contraction (30.4 ms after the onset of stimulation), permitting insufficient time for the accelerator muscle to develop peak force (Fig. 3B). By delaying projection, maximal acceleration and velocity may be substantially enhanced relative to a system in which projection is not delayed.

Several authors have hypothesized a preloading mechanism whereby the hyoglossi muscles hold the tongue on the entoglossus while the accelerator muscle develops tension (Zoond, 1933; Altevogt and Altevogt, 1954; Bell, 1989, 1990). However, electromyographic data indicate no activity in the hyoglossi prior to tongue projection (Wainwright and Bennett, 1992). Delayed projection observed in our accelerator muscle preparations provides an alternative preloading mechanism which, rather than invoking antagonistic muscle activity, depends on shape changes in the accelerator that permit the muscle to develop peak forces before tongue projection begins.

Electromyographic data indicate that the accelerator muscle becomes electrically quiescent about 11 ms prior to the onset of tongue projection (Wainwright and Bennett, 1992). The function of this pre-projection offset in activity is unknown, but contractile data indicate that the accelerator muscle maintains 100 % of peak pressure for at least 30 ms following stimulus offset and 90 % of peak pressure for 154 ms (Table 2, Fig. 4). Thus, electrical quiescence of the accelerator is accompanied by peak mechanical levels through the period of tongue projection.

Evolution of the chameleon tongue

The chamaeleonid tongue shares numerous features with the tongue of agamid lizards, the clade thought to be the sister taxon to the Chamaeleonidae (Estes *et al.* 1988). Agamid lizards possess a less-developed accelerator muscle, which appears to enable these lizards to translate the tongue along the entoglossus (Smith, 1988; Schwenk and Bell, 1988). During lingual prey capture, agamids protract the tongue beyond the gape while the animal lunges towards the prey (Smith, 1988; Schwenk and Bell, 1988; Schwenk and Throckmorton, 1989; Kraklau, 1990), although these lizards lack a supercontracting hyoglossi muscle (retractor muscle) and are unable to project the tongue off the entoglossus.

The agamid entoglossus is heavily tapered along most or all of its length (Gnanamuthu, 1937; Tanner and Avery, 1982; Smith, 1988; Schwenk and Bell, 1988; Kraklau, 1990) and contraction of the accelerator muscle presumably initiates immediate anterior translation of the tongue along the entoglossus. This anatomical condition is in marked contrast to the entoglossus found in *Chamaeleo jacksonii*, which is longer than the agamid process and is only tapered along the anterior 9 % of its length. We propose that the elongate, parallel-sided shape of the entoglossus is a key, derived feature of the chameleon feeding mechanism, and that this novel shape interacts with the accelerator muscle to provide a preloading mechanism enabling the accelerator muscle to generate maximal forces, thus enhancing the potential acceleration and velocity of the tongue during ballistic prey-capture behaviour.

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