

THE MECHANISM OF TONGUE PROJECTION IN CHAMELEONS

I. ELECTROMYOGRAPHIC TESTS OF FUNCTIONAL HYPOTHESES

By PETER C. WAINWRIGHT* AND ALBERT F. BENNETT

*Department of Ecology and Evolutionary Biology, University of California,
Irvine, CA 92717, USA*

Accepted 23 March 1992

Summary

In this paper we document the activity of key muscles of the tongue, hyobranchial apparatus and head during prey capture in the lizard *Chamaeleo jacksonii* Boulenger and use these data to test current hypotheses of chameleon tongue function. Electromyographic recordings were made during 27 feedings from nine individuals and synchronized with high-speed video recordings ($200 \text{ fields s}^{-1}$), permitting an assessment of the activity of muscles relative to the onset of tongue projection, contact between tongue and prey, and tongue retraction. Four major results were obtained. (1) The hyoglossi muscles exhibit a single burst of activity that begins between 10 ms before and 20 ms after the onset of tongue projection and continues throughout the period of tongue retraction. (2) The accelerator muscle exhibits a biphasic activity pattern, with the first burst lasting about 185 ms and ending an average of 10.6 ms prior to the onset of projection. (3) The accelerator muscle shows regional variation in morphology that corresponds with variation in motor pattern. The anterior region of the muscle, unlike the posterior portion, exhibits only a single burst of activity that begins 2.5 ms after the onset of tongue projection and is thus not involved in launching the tongue. (4) The geniohyoidei, sternohyoidei, sternothyroidei, depressor mandibulae, adductor mandibulae and pterygoideus all exhibit activity patterns consistent with previously reported kinematic patterns and their proposed roles. The major implications of these results for models of the chameleon feeding mechanism are (1) that the hyoglossi do not act to hold the tongue on the entoglossal process during a loading period prior to tongue projection, and (2) that the presence of 185 ms of intense activity in the accelerator muscle prior to tongue projection suggests the presence of a preloading mechanism, the nature of which is the subject of the companion paper.

Introduction

Chameleons feed by projecting their tongues over a full body length out of the

*Present address: Department of Biological Science, B-157, Florida State University, Tallahassee, FL 32306-3050, USA.

Key words: *Chamaeleo jacksonii*, chameleon, contractile kinetics, electromyography, feeding, lizard, skeletal muscle, tongue.

mouth, capturing prey on a sticky tongue pad. This remarkable behaviour has interested researchers for over a century, resulting in numerous hypotheses of the mechanism of ballistic tongue projection (Houston, 1828; Duvernoy, 1836; Brücke, 1852; Kathariner, 1894; Gnanamuthu, 1930; Zoond, 1933; Altevogt and Altevogt, 1954; Gans, 1967). However, with the exception of experiments performed by Zoond (1933), no direct observations have been made on muscle function. In particular, no data are available on the pattern of muscle activity associated with chameleon feeding. In this paper we test hypotheses of the chameleon tongue-projection mechanism with the first electromyographic recordings from key muscles of the tongue, hyobranchial apparatus and head of *Chamaeleo jacksonii*.

Early hypotheses of tongue function included rapid filling of the tongue by air or blood (Houston, 1828), launching of the tongue by a bone and muscle cross-bow analogue (Dewevre, 1895) and launching by rapid protraction of the hyoid skeleton (Duvernoy, 1836). Detailed anatomical observations made by Brücke (1852) led him to identify the accelerator muscle as the primary agent of propulsive forces in the system. This proposal was confirmed by Zoond (1933), whose classic experimental work demonstrated the ability of the sphincter-like accelerator muscle to force the tongue off the hyobranchial skeleton by squeezing on the tapered tip of the entoglossal process. The extreme extensibility of the chameleon hyoglossi muscles accommodates tongue projection and may limit the distance over which the tongue can be extended (Rice, 1973).

Currently, there are two contrasting views of the tongue projection mechanism. First, several authors have hypothesized a loading mechanism that holds the tongue on the entoglossal process while the accelerator muscle contracts, building up stress (Zoond, 1933; Altevogt and Altevogt, 1954; Bell, 1989, 1990). These authors proposed that the paired hyoglossi muscles, which attach the accelerator muscle and tongue pad to the base of the hyobranchial skeleton, contract and hold the tongue on the entoglossal process, until the moment of tongue projection, when they relax and allow the action of the accelerator muscle on the entoglossal tip to force the tongue away from the mouth towards the prey. The second view (Brücke, 1852; Gans, 1967) proposes no action of the retracting hyoglossi muscles until after the tongue has been projected. Under this hypothesis, tongue projection is accomplished directly by contraction of the accelerator muscle. The precise timing of accelerator activation has not been addressed by these authors (Brücke, 1852; Gans, 1967).

These competing hypotheses lead to different predictions regarding the patterns of activity in the accelerator and hyoglossi muscles. Under the first hypothesis (illustrated in Bell, 1989; Fig. 11) the accelerator muscle is predicted to have a single, long activity period beginning well before tongue projection and ending some time after tongue projection. This early onset of activity would correspond to the time when the hyoglossi muscles prevent tongue projection as the accelerator muscle builds up maximal force against the entoglossal process. Under this hypothesis, the hyoglossi muscles are predicted to show two distinct periods of

activity, one beginning and ending before tongue projection, and a second burst beginning sometime after tongue projection and corresponding to tongue retraction. The second hypothesis predicts a single accelerator burst beginning with or before the onset of tongue projection and only one hyoglossal muscle burst. This hyoglossal burst should begin after tongue projection and correspond to tongue retraction.

The primary purpose of this study is to test these hypotheses by documenting muscle activity patterns from chameleons during feeding. Electromyographic recordings made from the accelerator and hyoglossi muscles were synchronized with high-speed video recordings ($200 \text{ fields s}^{-1}$) to test the functions of these muscles during tongue projection. (1) Does the accelerator muscle show a single burst, and if so, does the onset of activity coincide with the onset of tongue projection or is it much earlier? (2) Do the hyoglossi muscles show a single burst of activity after tongue projection, or do they show a biphasic pattern with a second burst occurring prior to tongue projection?

In addition, we describe the motor activity of several other cranial and hyobranchial muscles that play important roles during prey capture. With these data we address three additional questions regarding the muscular basis of prey capture in chameleons. (1) Do the activity patterns of two jaw adductors and the jaw depressor correspond with periods of the gape cycle? (2) Is there regional variation in activity along the length of the accelerator muscle? (3) Is the activity of the geniohyoidei muscles consistent with their proposed role in rapidly protracting the hyobranchial apparatus at the onset of tongue projection (Brücke, 1852; Altevoigt and Altevoigt, 1954; Wainwright *et al.* 1991)?

Materials and methods

Specimens

The specimens of *Chamaeleo jacksonii* used in this study were collected during March 1989 in Nairobi, Kenya (under permit no. OP.13/001/18c94/19 to A.F.B.). Animals were brought to the University of California, Irvine, where they were housed indoors with a 12 h:12 h light:dark cycle and offered water and food (crickets) regularly. An incandescent bulb in each cage provided the animals with a thermal gradient from which they could select body temperature. Anatomical observations were made on five adult individuals (three females, two males; 93–121 snout–vent length, SVL) that had been preserved in 10 % formalin and stored in 70 % ethanol. Experimental individuals were trained under filming lights to take crickets held in a clip mounted at the end of a long wooden dowel.

Electromyographic recordings

Electromyographic data were collected from nine additional individuals (mean SVL=107.8 mm, s.e.=2.26). Animals were gradually anaesthetized with Halothane gas administered in a closed container. Bipolar electrodes were prepared from

paired 125 cm segments of 0.051 mm diameter insulated steel alloy wire. The wires were glued together for 15 cm at the recording ends and the insulation was scraped away from the electrode tips to expose 0.5 mm of electrode wire. These tips were threaded through the back of a 26 gauge hypodermic needle and the last 2 mm was bent back as they emerged from the needle barrel. Electrodes were implanted percutaneously into each muscle belly.

Recordings were obtained from the left-side member of six bilaterally paired muscles, in addition to the hyoglossi and accelerator muscles (Figs 1 and 2). Several muscles connect the hyobranchial skeleton to regions of the mandible, sternum and skull. These muscles provide the basis for extensive movements of the hyoid skeleton during feeding behaviour (Wainwright *et al.* 1991). Principal among these are the geniohyoidei (GH), which connect the mandibular symphysis to the basihyal and the ceratobranchials (Fig. 1A), the sternohyoidei (SH), which connect the posterior region of the basihyal to the sternum, and the sternothyroidei (ST), which attach the sternum to the distal tip of the ceratobranchials. These muscles are positioned appropriately to protract and retract the hyobranchial apparatus respectively. Distally the GH muscle separates into a lateral GH that inserts on the ceratohyals and ceratobranchials and a medial GH that inserts at the base of the entoglossal process (Fig. 1B). All recordings were made from the fused portion of the muscle. Lower jaw depression can be accomplished by the depressor mandibulae (DM), while numerous muscles may be used in jaw adduction (Rieppel, 1981). Among these, the pterygoideus (PT) inserts directly on the mandible while the adductor mandibulae medialis (AM) insert on the major tendon of the coronoid process.

A single ground electrode was implanted into the epaxial body musculature of each animal. Most implants passed through the animal's skin into the target muscle following landmarks previously determined by dissection. As the electrodes emerged they were fastened to the animal's skin under pieces of tape lined with a cyanoacrylate adhesive and bundled together into a cable that was glued to the animal's neck mid-dorsally. At the termination of five of the nine experiments, the animals were killed with an overdose of Halothane, fixed in formalin, and electrode placement was confirmed by dissection.

Implants into the accelerator and hyoglossi muscles were made by first gently pulling the animal's tongue out of its mouth and inserting electrodes directly into the muscles. Electrodes placed in the retractor muscle were always positioned near the left-side muscle's midpoint to minimize the danger of electrical interference from nearby muscles, in particular the accelerator muscle. Accelerator muscle implants were positioned in one of three locations. Most were in the posterior one-third of the muscle (Fig. 2, region 1). In two individuals each, additional electrodes were placed in either the middle one-third of the accelerator, where it still forms a complete ring around the entoglossal process (Fig. 2, region 2), or in the anterior-most section of the muscle, where it does not form a complete ring around the entoglossal process (Fig. 2, region 3). The hyoglossi and accelerator muscle electrodes passed through the opening of the mouth and were not fastened

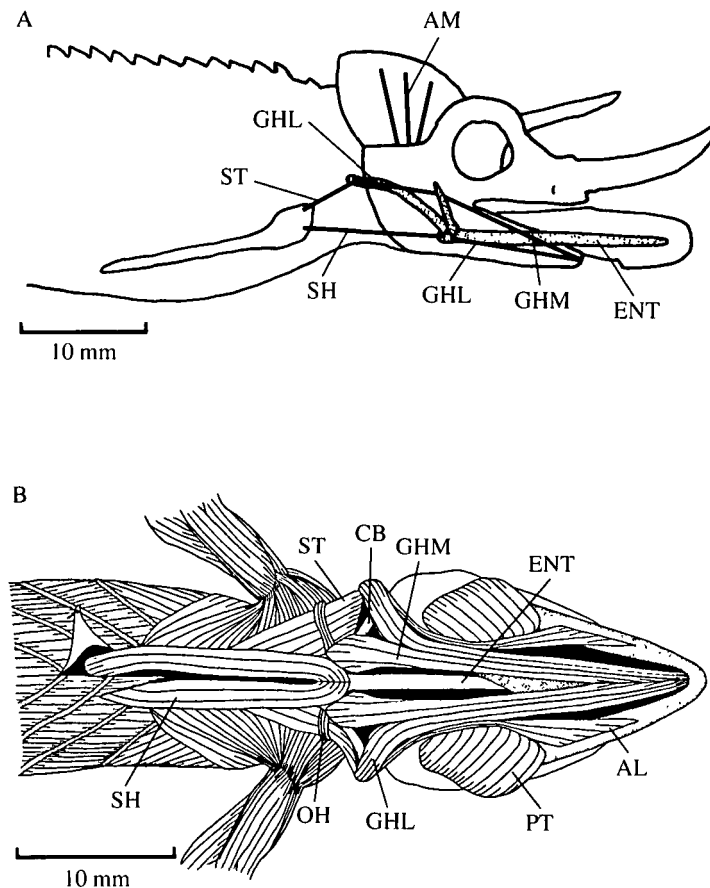


Fig. 1. Diagram of the hyolingual system of *Chamaeleo jacksonii* illustrating the positions of some of the muscles studied. (A) Schematic lateral view showing the animal in the late protraction phase immediately prior to tongue projection. Thick black lines indicate muscles from which electromyographic (EMG) activity was recorded in this study and their attachments. (B) Ventral view of the throat region emphasizing the muscles that attach the hyobranchial apparatus to other parts of the body. AL, adductor inferior labii; AM, adductor mandibulae medialis; CB, ceratobranchial; ENT, entoglossal process; GHM, geniohyoideus medialis; OH, omohyoideus; PT, pterygoideus; SH, sternohyoideus; ST, sternothyroideus.

to the animal's dorsum. These electrodes could be projected freely with the tongue during prey capture attempts.

Electrical signals from up to five muscles were amplified 5000 times with Grass P511K signal conditioners set with a low bandpass of 100 Hz and a high bandpass of 3000 Hz. The 60 Hz notch filter was always used. Signals were recorded on a Hewlett Packard 4086 eight-channel FM instrumentation recorder and later played back on a Gould 3600 chart recorder at one-eighth the recorded speed.

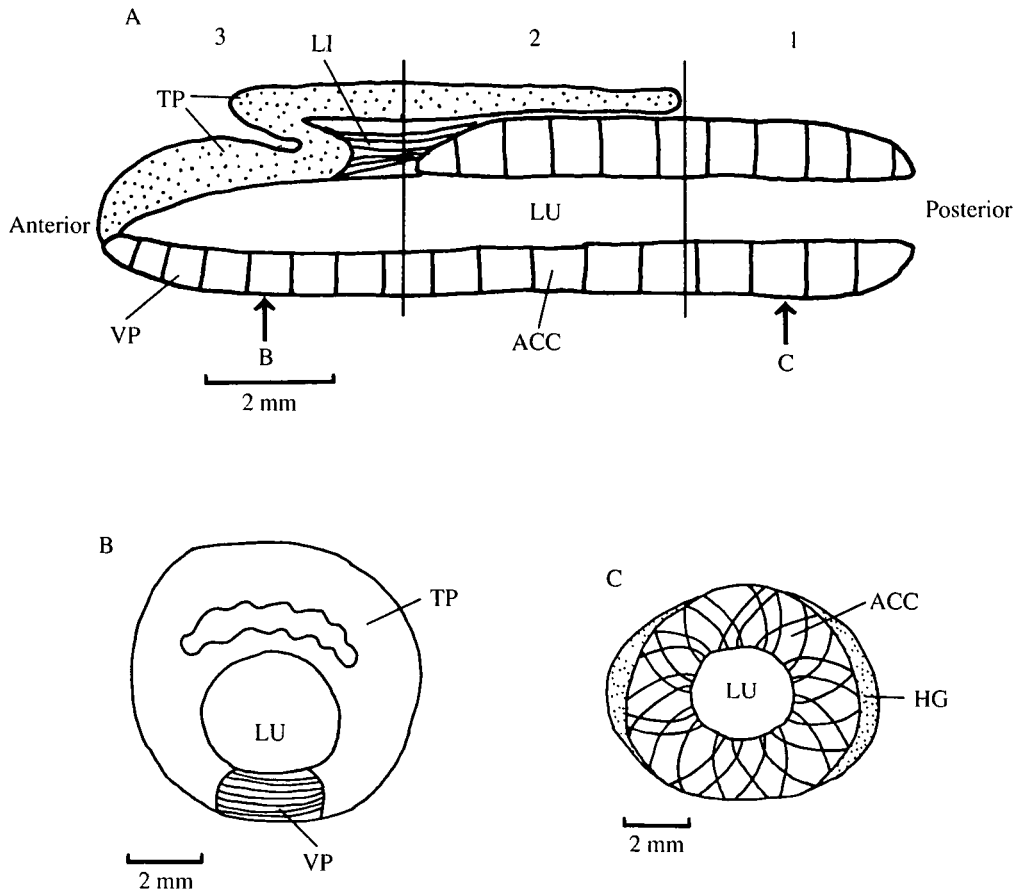


Fig. 2. Schematic diagrams of the accelerator muscle and tongue pad. (A) Parasagittal section through tongue pad from a 112 mm snout-vent length *Chamaeleo jacksonii* fixed with the tongue on the entoglossal process. The numbers 1, 2 and 3 indicate the regions of the accelerator muscle from which electromyograms were recorded. Arrows indicate the approximate positions of the cross sections shown in B and C. Note that the region of the accelerator that forms a complete ring around the lumen includes only about the posterior 60% of the muscle. (B) Cross section through the anterior region of the tongue illustrating that the accelerator is reduced to a ventral extension here with fibres oriented transversely. (C) Cross section through the posterior region of the accelerator muscle indicating the complete ring that this muscle forms around the lumen. Overall fibre orientations are indicated but appear in sections as interleaved sheets in transverse planes (see, for example, Gnanamuthu, 1930; and photographs of sections in Bell, 1989). The hyoglossi muscles run along the sides of the accelerator muscle and insert on the tongue pad. ACC, accelerator muscle; HG, hyoglossal muscle; LI, longitudinal adductor muscles; LU, lumen; TP, membrana glandulosa of the tongue pad; VP, ventral projection of the accelerator muscle.

Recording sessions took place with animals perched on a wooden dowel in front of a light background marked with a 1.0 cm grid. Animal body temperatures ranged between 21 and 24°C. High-speed videos (200 fields s⁻¹) were recorded with a NACHSV 400 system using one strobe for lighting. Crickets (*Acheta domesticus*) were presented in a clip and animals readily attempted to feed in lateral view of the camera. The output of a signal generator operating at 100 Hz was recorded on one channel of the FM tape recorder and simultaneously on the video screen to synchronize the kinematic and electromyographic records.

Electromyographic analysis

Timing of muscle activity relative to tongue projection kinematics was established for each feeding sequence. The onset of tongue projection was defined as time=0 and was used as the reference time for most timing measures. Forward movements of the tongue occurred during the protraction phase prior to the strike and during tongue projection. Therefore, the onset of tongue projection was identified as the first video field in which the tongue had travelled more than 5 mm from the previous frame (Wainwright *et al.* 1991). Three additional kinematic variables were measured from the video record of each feeding. First, the time from the onset of tongue projection until the tongue first contacted the prey was measured to the nearest 5 ms. Second, the time from the onset of tongue projection until the tongue was retracted within the margin of the gape was measured to the nearest 5 ms. Third, the maximum distance that the tongue travelled during each feeding was measured in millimetres. Here and throughout this study, tongue distance was measured as the linear distance between the intersection of the entoglossus and a line defined by the anterior margins of the upper jaw and the mandible, and the distal tip of the tongue. Maximum tongue distance was usually longer than the distance from the chameleon to the prey because the tongue normally continued for several millimetres after the initial prey contact (Wainwright *et al.* 1991).

To quantify the timing of muscle activity relative to kinematic events, the onset of tongue projection was marked on the chart recordings of each feeding and used as the reference from which activity onset or offsets were measured. For each muscle, the duration of each activity burst that occurred during the strike was measured to the nearest 1.25 ms and for most muscles the onset time of bursts was measured relative to the onset of tongue projection (time=0), to the nearest 1.25 ms. Onset times of each muscle were defined as periods when (1) muscle activity reached three times the amplitude of that channel's background noise level, and (2) activity was sustained for at least 20 ms. Thereafter, activity was considered continuous if quiescent periods did not exceed 30 ms. Several muscles had more than one activity burst during the course of the feeding sequence. For the first accelerator muscle burst, the first depressor mandibulae burst, the first geniohyoideus burst and the sternothyroideus burst, timing was measured from the offset of the burst to time=0. Because the activity of the retractor muscle relative to contact between the tongue and prey was also of interest, an additional

variable measured the time between the onset of retractor activity and prey contact. Twenty-seven EMG variables were measured from a total of 27 feedings from nine individuals. The largest sample for a single muscle was 18 for both the accelerator muscle region 1 and the hyoglossus muscle. The number of feedings per individual ranged from two to five.

To compare the motor patterns observed for the three regions of the accelerator muscle, a series of two-way analyses of variance (ANOVA) were run. Although data for electrode region 1 were collected from six individuals, data for the other two regions were obtained from only two individuals each. In experiments in which data were obtained from regions 2 or 3, data were also obtained from region 1. Data were recorded from at least two accelerator regions in every preparation. The design for each two-way ANOVA crossed individuals (a random factor) with electrode position (a fixed factor). In each case there were two individuals and two electrode positions. The *F*-ratios used to test the significance of the electrode region effect were constructed with the region effect mean squares in the numerator and the interaction term mean squares in the denominator (Sokal and Rohlf, 1981).

Results

Anatomy

The morphology of the chameleon head has been described in detail elsewhere and only the salient features necessary for a discussion of the tongue projection mechanism are provided here. The reader is referred to other papers for more detailed accounts of cranial musculature (Mivart, 1870; Rieppel, 1981), hyobranchial structures (Mivart, 1870; Gnanamuthu, 1930, 1937; Tanner and Avery, 1982; Schwenk and Bell, 1988) and lingual morphology (Gnanamuthu, 1930; Gans, 1967; Schwenk and Bell, 1988; Bell, 1989).

The muscular tongue of *C. jacksonii*, as in other chameleons, is mounted on an elongate entoglossal process (Figs 1 and 2; Gnanamuthu, 1930, 1937). From anterior to posterior, the tongue is formed by a large tongue pad, the accelerator muscle and the hyoglossi muscles, which attach the base of the hyobranchial skeleton to the posterior end of the 'tongue knob' (=accelerator muscle plus tongue pad). The accelerator muscle is cylindrical in shape with a lumen running longitudinally through its centre (Fig. 2). The entoglossal process fits into this lumen. Along much of its length the accelerator forms a complete muscular ring around the lumen. However, a key feature of the accelerator muscle is that its most anterior section (about 35 % of its total length) does not form a ring around the lumen and is present only ventrally as an anterior extension (Fig. 2; see also Gnanamuthu, 1937; Bell, 1989). At rest, the entoglossal process of a 111 mm SVL male *C. jacksonii* protrudes 6 mm beyond the end of the 10 mm long circular portion of the accelerator muscle.

The muscle fibres of the accelerator muscle are in the plane of a transverse

section, and project radially at an angle from the inner lumen wall to the inner side of the outer skin of the muscle (Fig. 2C; Gnanamuthu, 1930; Gans, 1967; Bell, 1989). This muscle is thought to act as a sphincter, and experimentally has been shown to squeeze on the entoglossal process during contraction (Zoond, 1933). Further support for this function is provided by pressure recordings made within the lumen during contraction of the accelerator muscle (Wainwright and Bennett, 1992).

Motor pattern

Kinematic profiles of gape distance and hyoid retraction were similar to those seen in unrestrained *C. jacksonii* (P. C. Wainwright and A. F. Bennett, unpublished observations), *C. oustaleti* (Wainwright *et al.* 1991) and *C. zeylanicus* (Bell, 1990), suggesting that the experimental application of anaesthesia and subsequent electrode implantation did not significantly affect these aspects of prey capture kinematics. Profiles of tongue projection, however, did show a slightly more abrupt deceleration profile as the tongue approached the prey and more rapid initial tongue retraction than seen in unencumbered chameleons (compare Figs 3–5 with Fig. 5 in Wainwright *et al.* 1991). Other phases of the tongue projection cycle (e.g. tongue protraction, the initial stages of tongue projection and most of the retraction phase) appeared unaffected.

Between the onset of tongue projection and prey contact (mean time=25.3 ms, s.e.=1.39 ms), the tongue moved rapidly towards the prey (Fig. 3). Projection distances were relatively modest in the experiments (i.e. approximately 60–100 % of SVL) and did not approach the maximum capability of individuals (mean projection distance=91.7 mm, s.e.=9.3 mm). We have observed that individual *C. jacksonii* can project their tongue as much as two snout–vent lengths out of their mouth (P. C. Wainwright and A. F. Bennett, unpublished observations). During projection the gape decreased slightly (see Fig. 5). Between prey contact and retraction of the tongue within the margin of the gape (mean time=337.1 ms, s.e.=43.2 ms), the gape rapidly opened as the hyoglossi muscles contracted and pulled the tongue knob and prey towards the mouth. During the latter half of this period, the hyoid became visible in the throat as it was retracted with the tongue (see Fig. 5). The jaws closed rapidly after the tongue and hyoid had been retracted within the margin of the gape.

The posterior regions of the accelerator muscle exhibited a distinct biphasic pattern of activity (Figs 3, 4 and 6; Table 1). The first activity burst lasted an average of 186.8 ms and ended 10.6 ms prior to the onset of tongue projection. There was considerable variation in the timing of activity offset in this burst relative to the onset of projection (e.g. compare Figs 3 and 4) but in 14 of 18 feeding sequences projection onset did not commence until after the muscle had ceased EMG activity. During the first burst, there was slight protraction of the tongue but no tongue projection (e.g. Figs 3 and 4). The second accelerator burst began 15.2 ms after the onset of tongue projection (10.1 ms before prey contact) and lasted an average of 566.4 ms. Thus, there was activity in the accelerator

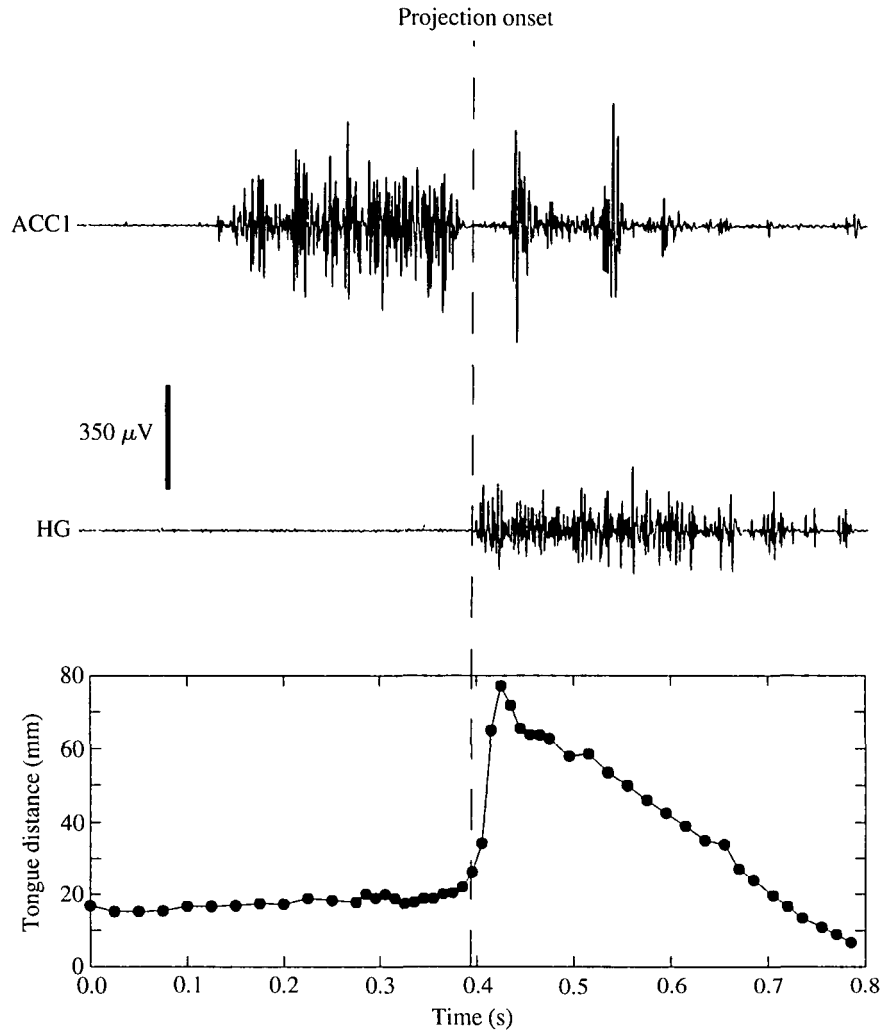


Fig. 3. Sample myograms with synchronized kinematic plot of distance of the tongue from the margin of the gape (=tongue distance 0) during prey capture in *Chamaeleo jacksonii*. Tongue distance from the margin of the gape was measured from video fields recorded at $200 \text{ fields s}^{-1}$. The vertical dotted line indicates the onset of tongue projection from the mouth. Region 1 of the accelerator muscle (ACC1) and the hyoglossal muscle (HG) are illustrated. Note (1) that the accelerator muscle is active for about 200 ms prior to the onset of tongue projection, (2) that the accelerator muscle is not active during tongue projection, and (3) that the hyoglossal muscle is not active prior to the onset of tongue projection. Contact of the tongue with the prey occurred at the time of maximal tongue extension.

throughout tongue retraction and in two feeding sequences we observed activity continuing through the first two cycles of chewing.

Analysis of variance revealed no differences in activity between regions 1 and 2 of the accelerator muscle (Table 2). The timing of both bursts relative to the onset

Table 1. Summary statistics (mean±standard error) for 27 EMG timing variables measured from recordings of nine muscles of the tongue, hyobranchial region and head of *Chamaeleo jacksonii*

Muscle	Duration (ms)	Relative timing* (ms)
Accelerator region 1 (18, 6), first burst	186.8±12.5	-10.6±4.9**
Accelerator region 1 (18, 6), second burst	566.4±53.2	15.2±5.8
Accelerator region 1 (5, 2), first burst	193.5±34.2	-9.6±7.8**
Accelerator region 2 (5, 2), second burst	594.7±47.6	18.3±7.1
Accelerator region 3 (6, 2)	505.0±49.3	2.5±10.5
Hyoglossal (18, 6)	565.7±29.0	-2.3±2.9
Geniohyoideus (16, 5), first burst	>1000	-109.9±18.5**
Geniohyoideus (16, 5), second burst	167.6±21.6	-49.9±6.7
Sternohyoideus (13, 4)	357.7±32.6	194.2±22.3
Sternothyroideus (4, 2)	>1000	-12.8±28.8**
Depressor mandibulae (15, 4), first burst	>1000	5.0±5.6**
Depressor mandibulae (15, 4), second burst	368.0±22.3	32.5±8.5
Pterygoideus (6, 3)	167.3±34.1	285.0±48.4
Adductor mandibulae medialis (7, 3)	82.4±23.2	235.7±52.1

* Relative timing variables, unless otherwise indicated, are time measured from the onset of tongue projection to the onset of the muscle burst.

** These relative timing variables are time measured from the onset of tongue projection to the offset of the muscle burst.

Numbers in parentheses following muscle names indicate the total number of strike sequences analyzed and the number of individuals from which sequences were obtained.

Table 2. Results of two-way ANOVAs contrasting electrode position effects on EMG variables in the accelerator muscle

EMG variable	Region effect*
Comparisons between regions 1 and 2	
Duration burst 1	1.23
Duration burst 2	0.73
Offset burst 1	0.81
Onset burst 2	1.03
Comparisons between regions 1 and 3	
Duration burst	2.11
Onset burst	1.98

* All *F*-ratios are not significant, $P>0.05$.

Entries are *F*-ratios from significance tests for the electrode position effect.

Comparisons between regions 1 and 3 contrast the second burst of position 1 with the only burst of region 3.

Onsets and offsets were measured relative to the onset of tongue projection.

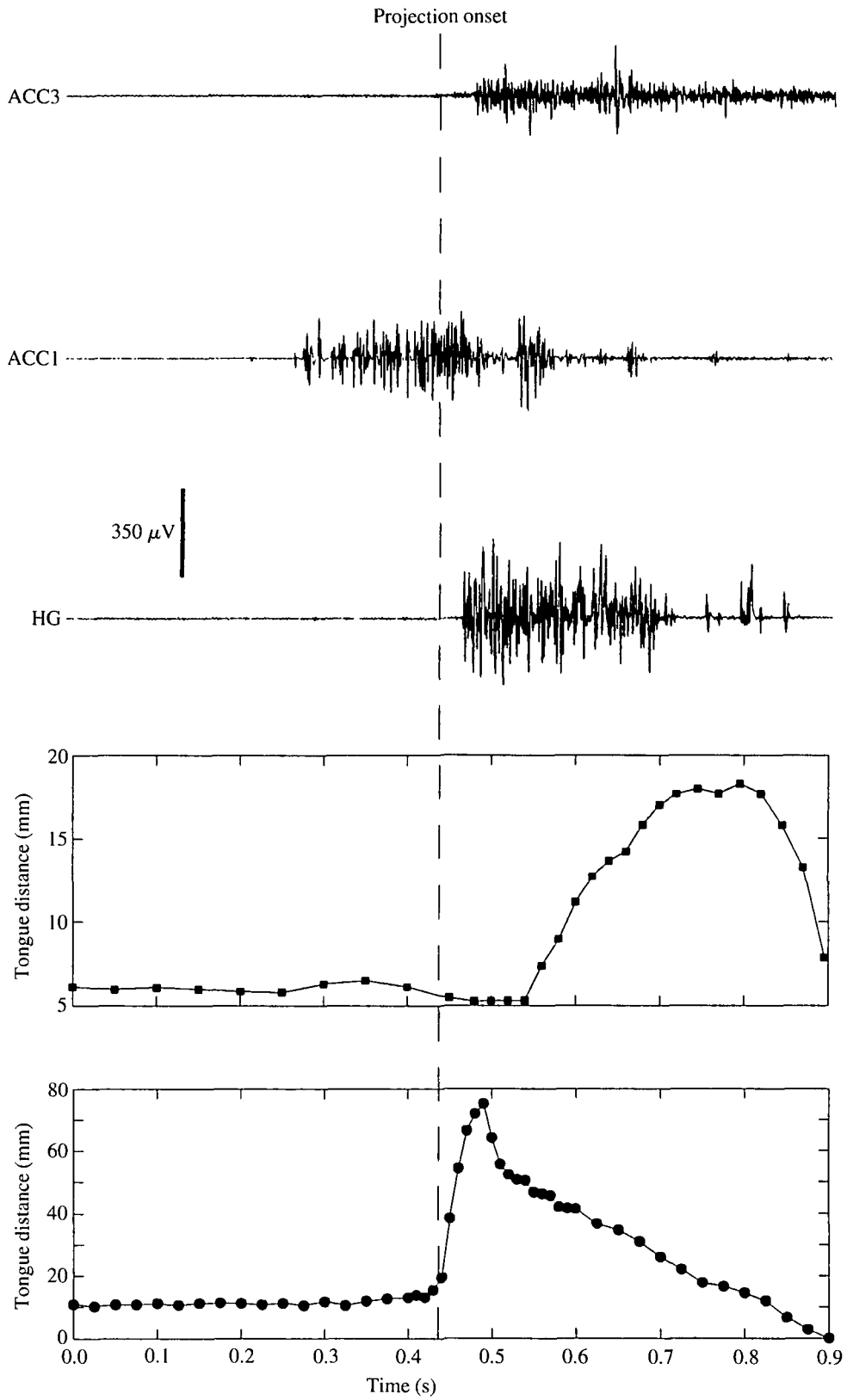


Fig.4

Fig. 4. Sample myograms and synchronized kinematic data of gape distance and tongue distance from the margin of the gape (=tongue distance 0) during prey capture in *Chamaeleo jacksonii*. Kinematic data were measured from video fields recorded at 200 fields s⁻¹. Regions 1 and 3 of the accelerator muscle (ACC1 and ACC3) and the hyoglossal muscle (HG) are illustrated. See Fig. 2 for anatomical locations of the electrode positions. Tongue distance was measured from the margin of the gape. Note that the two positions of the accelerator muscle show different activity patterns. Contact of the tongue with the prey occurred during the video field immediately prior to maximal tongue extension.

of tongue projection and the duration of each burst did not vary along the length of the region of the accelerator muscle that forms a ring around the entoglossal process. However, there was a clear qualitative difference between the posterior regions of the accelerator and the most anterior region (Fig. 4). Accelerator muscle region 3 never showed the first activity burst characteristic of the posterior regions. The single activity burst from this region began 2.5 ms after the onset of tongue projection (26.4 ms before prey contact) and lasted 505 ms. The onset of activity in region 3 occurred before the onset of activity in the second burst of region 1 in all six of the feeding sequences analyzed, yet because of the variation in these times they were not statistically distinguishable (Table 2).

The hyoglossi exhibited a single burst of activity that began 2.3 ms before the onset of tongue projection, and 22.0 ms prior to prey contact. This activity burst had an average duration of 565.7 ms, and lasted throughout the period of tongue retraction (Figs 3 and 4).

The geniohyoideus muscle showed two distinct periods of activity during the tongue projection sequence (Figs 5 and 6; Table 1). The first activity period began with the earliest stages of tongue protraction and continued intermittently until 109.9 ms prior to the onset of tongue projection. The tongue protraction phase was highly variable in duration, lasting from 1 s to over 3 min (see also Bell, 1990; Wainwright *et al.* 1991), so the actual duration of this activity burst in the geniohyoideus was not measured. The second activity burst began 49.9 ms prior to tongue projection and lasted 167.6 ms, thus overlapping with the time of tongue projection and prey contact (Figs 5 and 6). In three of the 16 feedings for which geniohyoideus data were obtained, there was a third activity burst (e.g. Fig. 5) that began 312 ms (s.e. = 27.9 ms) after the onset of tongue projection and lasted for an average of 71 ms (s.e. = 32.4 ms).

The sternohyoideus exhibited a single activity burst that commenced 194.2 ms after the onset of tongue projection and lasted 357.7 ms (Table 1; Figs 5 and 6). This burst overlapped the period of hyoid retraction that occurred once the tongue had been mostly retracted into the mouth (Fig. 5). The sternothyroideus showed a single, long burst of activity that corresponded to the first geniohyoideus burst (Fig. 6). Activity began early in the slow protraction phase at the time of feeding and continued at a low level until 12.8 ms before the onset of projection.

The depressor mandibulae showed intermittent activity beginning with the onset of the tongue protraction phase until its offset at 5 ms after the onset of

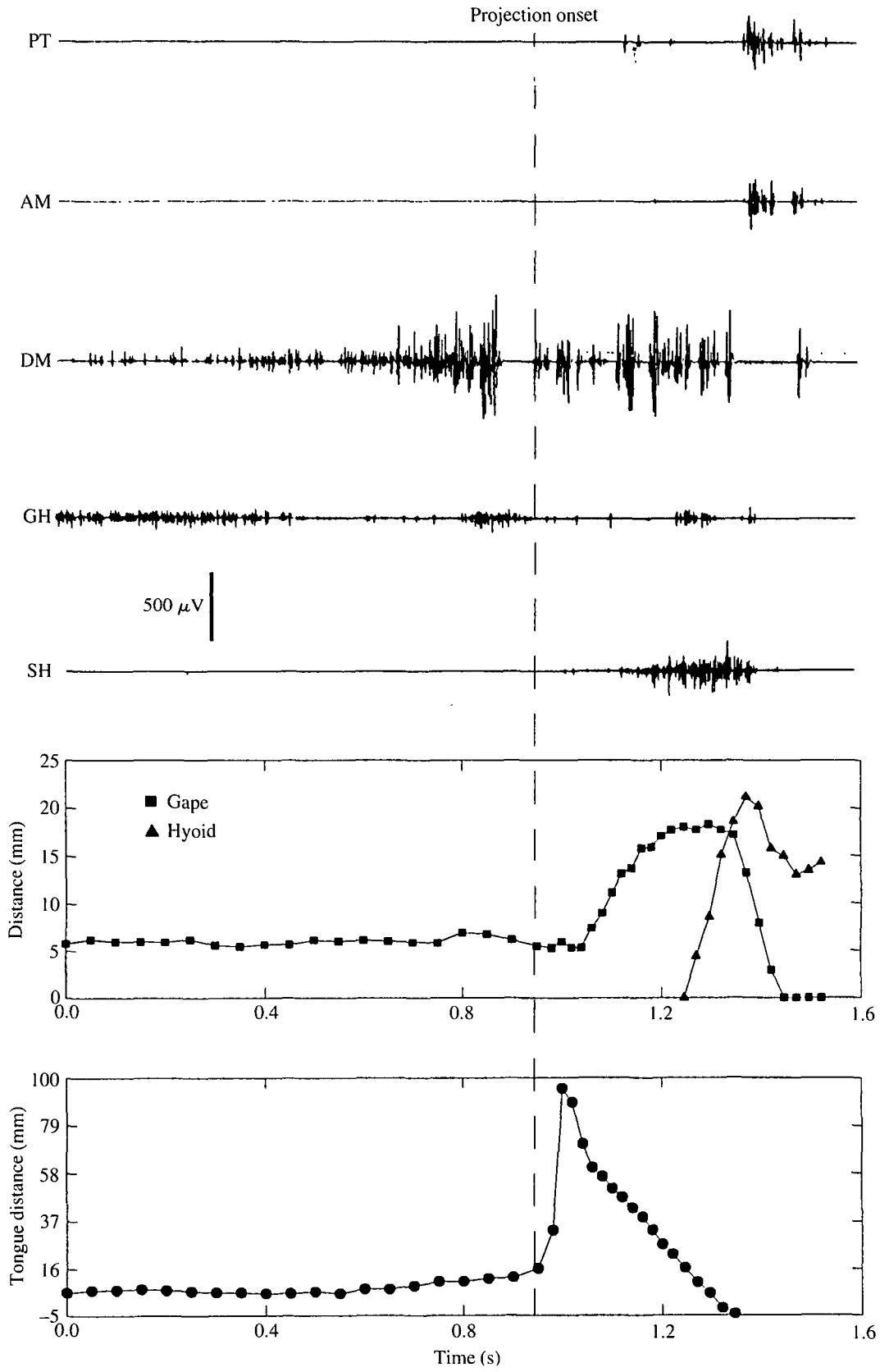


Fig. 5

Fig. 5. Sample electromyograms of five muscles during tongue projection in *Chamaeleo jacksonii* with synchronized kinematic data from the head, hyoid and tongue. Kinematic data were measured from fields of a $200 \text{ fields s}^{-1}$ video. Variables shown are the distance of the anterior tip of the tongue to the margin of the gape, the gape distance between the tips of the jaws, and the distance of the hyobranchial apparatus in the throat region from the vertex of the jaws. Increasing values of hyoid position indicate that it is being retracted. Tongue distance was measured from the margin of the gape. Contact of the tongue with the prey occurred at the time of maximal tongue extension. PT, pterygoideus; AM, adductor mandibulae medialis; DM, depressor mandibulae; GH, geniohyoideus; SH, sternohyoideus.

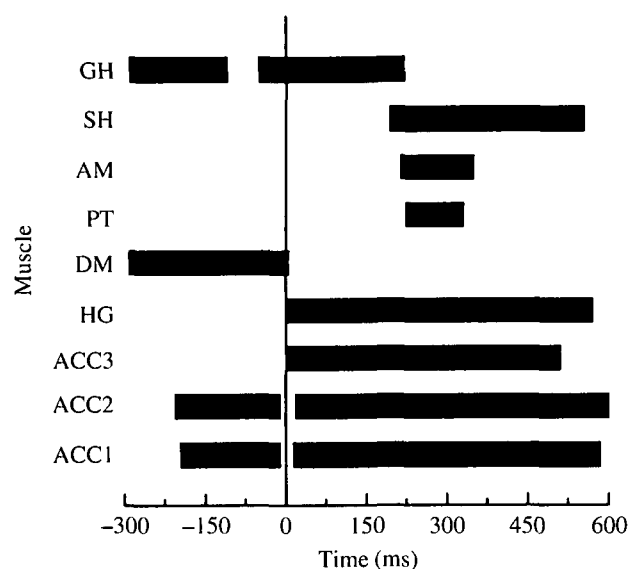


Fig. 6. Summary EMG bar diagram for nine muscles and electrode positions recorded from the head region of *Chamaeleo jacksonii* during prey capture. Bars indicate the mean duration of activity bursts and activity time relative to the onset of tongue projection (time=0). Data and sample sizes for each muscle are given in Table 1. The onsets of the first bursts of the depressor mandibulae (DM) and the geniohyoideus (GH) and the only sternohyoideus burst are not shown because these muscles became active more than 1.0 s prior to tongue projection. All bursts shown occurred in 100 % of all feeding sequences analyzed. SH, sternohyoideus; AM, adductor mandibulae medialis; PT, pterygoideus; HG, hyoglossal muscle; ACC1–3, accelerator muscle regions 1–3.

tongue projection (Fig. 5). A second burst began 32.5 ms after the onset of tongue projection and continued for 368 ms, thus overlapping the period of gape increase that occurred during the tongue retraction phase.

The two jaw adductors, the pterygoideus and the adductor mandibulae medialis, showed very similar activity patterns (Figs 5 and 6). A single burst characterized these muscles during the strike, with activity beginning 285 ms after the onset of

tongue projection in the pterygoideus and 235.7 ms after the onset of tongue projection in the adductor mandibulae. The burst lasted an average of 167.3 ms in the pterygoideus and 82.4 ms in the adductor mandibulae (Table 1).

Discussion

Our electromyographic data from *C. jacksonii* provide a quantitative picture of muscle activity patterns during tongue projection and permit critical tests of previous proposals for the roles of various muscles. The central conclusions of this study are as follows. (1) The hyoglossi exhibit a single burst of activity that commences after the onset of tongue projection, thus ruling out the role of this muscle in actively restraining the tongue on the entoglossal process while the system is loaded by the accelerator muscle. (2) The accelerator muscle exhibits a distinct biphasic activity pattern, with the first burst lasting about 185 ms and ending 10.6 ms before the onset of tongue projection. This pattern demonstrates that the action of this muscle in projecting the tongue does not occur synchronously with the onset of the muscle's contraction, suggesting the presence of a loading mechanism, although one that is not dependent on hyoglossi restraint. (3) The accelerator muscle shows regional variation in activity pattern. The posterior region, which forms a complete ring around the entoglossal process, exhibits a biphasic pattern, while the anterior region, which occurs as an anterior projection of the muscle ventral to the entoglossal process, shows only a single burst of activity that begins after the onset of tongue projection. (4) The geniohyoideus muscle shows a burst of activity that overlaps with the period of tongue projection, supporting its previously proposed role in rapidly protracting the hyobranchial apparatus during the onset of tongue projection. We discuss these results in turn, with emphasis on their implications for models of tongue function in *Chamaeleo*.

Hyoglossal muscle

The central distinction between the two current models of the chameleon tongue projection mechanism concerns the role of the hyoglossi muscles. One model (Zoond, 1933; Altevogt and Altevogt, 1954; Bell, 1989, 1990) predicts a biphasic pattern of activity in this muscle, with the initial burst being involved in holding the tongue on the entoglossal process while accelerator forces are developed. The second model implicates only a single burst of hyoglossal activity, during the period of tongue retraction (Brücke, 1852; Gans, 1967). Our results from *C. jacksonii* show clearly (1) that there is only a single burst of hyoglossal activity, and (2) that the onset of this burst occurs between 10 ms before and 20 ms after the onset of tongue projection and continues through tongue retraction. This activity is preceded by burst activity in the accelerator muscle by approximately 200 ms. This result implies that the function of the hyoglossi muscles is to decelerate and retract the tongue following projection and not to permit preloading of the tongue prior to projection.

Accelerator muscle

The accelerator muscle shows two distinct morphological regions (Fig. 2). Anteriorly the muscle is present only as a forward projection ventral to the entoglossal process, while the posterior 60–65 % of the muscle forms a complete ring around the entoglossal process. It is this posterior region that has been shown to contract in a sphincter-like fashion, squeezing on, and forcing the tongue off, the entoglossal process (Zoond, 1933; Wainwright and Bennett, 1992). The function of the anterior region of the accelerator muscle is not known.

The posterior region of the accelerator muscle showed a biphasic activity pattern, with the first burst lasting about 185 ms and ending 10.6 ms before the onset of tongue projection. Tongue projection occurred during a quiescent period and was followed by a second burst, beginning 15.2 ms after the onset of tongue projection and continuing throughout tongue retraction. Although the accelerator muscle is not electrically active during the period of actual tongue projection, we show elsewhere (Wainwright and Bennett, 1992) that mechanically the muscle is probably exerting maximal force throughout projection. Thus, the period of activity in the accelerator muscle that powers tongue projection begins about 200 ms before projection occurs. This pattern implies that some mechanism prevents the accelerator muscle from projecting the tongue while the muscle actively generates tension for about 0.2 s (Figs 3 and 4). The nature of this mechanism is the subject of the companion paper (Wainwright and Bennett, 1992).

The activity of the accelerator muscle observed in *C. jacksonii* is essentially that predicted by a model of tongue function that implicates a loading mechanism (Zoond, 1933; Altevogt and Altevogt, 1954; Bell, 1989, 1990). Although the hyoglossi muscles are not the agent that prevents tongue projection during the protraction phase, it is clear that the accelerator is strongly active for a considerable time before tongue projection occurs. The alternative model (Brücke, 1852; Gans, 1967) accurately predicts hyoglossal activity but does not specifically predict the early onset of activity in the accelerator muscle.

What is the implication of the 25 ms (mean=24.6 ms, s.e.=6.1 ms) period of electrical inactivity that occurs in the accelerator muscle during the time of tongue projection (Fig. 3)? Little, if any, loss of mechanical tension occurs in the muscle during this period (Wainwright and Bennett, 1992). It therefore seems possible that the pause in activity may not serve a mechanical function *per se*, but rather occurs as a result of some other event. Since this period of inactivity usually begins before the tongue is launched, the abrupt projection action itself is not the cause of accelerator inactivity. Nevertheless, the temporal connection between tongue projection and accelerator quiescence does suggest that the two are related in some way. Another possibility is that this is a feature of the motor pattern that has been retained from an ancestral condition. Unfortunately, no lingual motor pattern data are available from other iguanian lizard taxa, specifically members of the Agamidae, the family thought to be the sister taxon to the Chamaeleontidae (Estes *et al.* 1988). Members of the Agamidae do possess an accelerator homo-

logue that functions in conjunction with hyobranchial structures in much the same way as the chameleon accelerator (Smith, 1988), although the tongue of these lizards lacks the extreme extensibility seen in chamaeleontids (Smith, 1988; Schwenk and Bell, 1988).

Another unexpected aspect of the accelerator activity pattern is the long period of activation that occurred once the tongue had been launched and continued throughout prey contact and tongue retraction. It may be that the muscle becomes stiff during contraction, providing a more rigid structure for the intrinsic tongue pad muscles to work against during initial contact with the prey and subsequent adhesion during retraction.

The anterior region of the accelerator muscle is not only morphologically distinct from the posterior portions but also shows a different pattern of use during tongue projection. No activity was seen in this region of the accelerator muscle prior to tongue projection, indicating that this region does not function as part of the tongue-projecting mechanism. However, this area of the muscle is active throughout prey contact and tongue retraction. Bell (1989) has shown that the fascia surrounding this region of the accelerator muscle is a site of attachment for several muscles that connect the tongue pad to the accelerator muscle. The tongue pad is known to change shape actively during projection and prey contact (Altevogt and Altevogt, 1954; Bell, 1990), and activity in this region of the accelerator may work in conjunction with activity in the posterior regions to establish a firm site of attachment for the tongue pad muscles.

One implication of the observed regional patterns of accelerator activity is that the muscle clearly does not 'contract smoothly in a wave from front to back' as has been suggested (Bramble and Wake, 1985). Rather, the pattern seen in *C. jacksonii* is that the posterior region is activated as a whole (Table 2) while the anterior region is activated after the posterior portions (Figs 4 and 6). Since there is no activity in the anterior portion prior to the onset of tongue projection, this region cannot contribute to launching of the tongue. It appears that all parts of the posterior, circular region of the accelerator act synchronously in powering tongue projection.

Geniohyoidei, sternohyoidei and sternothyroidei

Several authors (Brücke, 1852; Zoond, 1933; Altevogt and Altevogt, 1954; Wainwright *et al.* 1991) have hypothesized that hyobranchial protraction assists the accelerator muscle during the onset of tongue projection. This proposal is supported by kinematic data that show rapid hyobranchial protraction during the onset of tongue projection (Wainwright *et al.* 1991). The only muscles positioned to protract the hyobranchial apparatus are the geniohyoidei (Fig. 1) and these muscles did show a 167.6 ms long burst of activity that began 49.9 ms prior to the onset of tongue projection. We note, however, that this burst of activity was typically not of high amplitude (Fig. 5), as one might expect given the rapidity of hyoid protraction. Nevertheless, the presence of this activity burst is consistent with available kinematic data (Wainwright *et al.* 1991) and supports the role of this

muscle in rapidly protracting the hyobranchial apparatus during the onset of tongue projection.

Prior to tongue projection, there is an initial activity period of the geniohyoideus (Figs 5 and 6) that begins with the onset of tongue protraction, a phase that can last for 2–3 min (Bell, 1990; Wainwright *et al.* 1991) and suggests that this muscle takes part in hyoid protraction.

During protraction, the hyobranchium unfolds and dorsally oriented ceratohyals and ceratobranchials move into an anterior–posterior orientation (Fig. 1; Altevogt and Altevogt, 1954; Wainwright *et al.* 1991). This unfolding action is accompanied by activity in the sternothyroidei (Table 1), which attach the tips of the ceratobranchials to the sternum. Thus, as the geniohyoidei protract the entoglossal process, the sternothyroidei pull back on the ceratobranchials. The effect is that the hyobranchial apparatus unfolds during initial hyobranchial protraction.

The sternohyoideus muscle was not active until the tongue had been nearly retracted back into the mouth (Fig. 5). At this time hyobranchial retraction began and was accompanied by sternohyoideus activity. The sternohyoideus is thus active during the only hyobranchial retraction that occurs during the tongue projection sequence.

Jaw depressor and adductors

The period of slow hyobranchial protraction was accompanied by low levels of activity in the depressor mandibulae, indicating slow opening of the mouth during this time (Fig. 5). As the onset of tongue projection approached, the activity level of the depressor appeared to increase significantly (e.g. Fig. 5). Interestingly, this increase in jaw depressor activity was not accompanied by an increase in gape, yet no antagonistic activity was seen in the two jaw adductors that were studied (Fig. 5). The depressor became abruptly inactive an average of 5.0 ms after the onset of tongue projection, and after this time the gape decreased slightly (Fig. 5). A slight decrease in gape immediately following the onset of tongue projection has been reported in numerous chameleon species (Bels and Baltus, 1987; Bell, 1990; Wainwright *et al.* 1991) and appears to be a general feature of the strike gape profile.

The slight decrease in gape is followed by a period of rapid gape increase, which is accompanied by depressor activity (Fig. 4). The time of maximum gape coincides with the period of hyobranchial retraction (Fig. 4; Wainwright *et al.* 1991). As the mouth then begins to close, depressor activity ceases and activity is seen in the two jaw adductors, the adductor mandibulae medialis and the pterygoideus. Activity of the jaw adductors was not seen at other times during the gape cycle.

We note the following three points in conclusion. (1) The two current hypotheses of chameleon tongue function focus on the actions of the accelerator and hyoglossi muscles. Neither hypothesis correctly predicts the activity pattern of both muscles, which we measured in this study. The long activity burst seen in the accelerator muscle prior to the onset of tongue projection does imply the presence

of a preloading mechanism, as predicted by one hypothesis (Zoond, 1933; Altevogt and Altevogt, 1954; Bell, 1989, 1990). However, the predicted function of the hyoglossi muscles in holding the tongue on the entoglossal process during this loading period was clearly refuted by our EMG data. The other hypothesis (Brücke, 1852; Gans, 1967) correctly predicted the activity pattern of the hyoglossi muscles but was incorrect in proposing that accelerator activity coincides with tongue projection. What is the mechanism that prevents the tongue from projecting during the long initial burst of accelerator activity and what is the trigger that releases the loaded system? These questions are addressed in our companion paper (Wainwright and Bennett, 1992).

(2) Activity patterns of those muscles connecting the hyobranchial apparatus to regions of the head and body closely matched expectations based on kinematic observations. The geniohyoideus, sternohyoideus and sternothyroideus all showed activity patterns consistent with previously observed hyobranchial movements (Altevogt and Altevogt, 1954; Wainwright *et al.* 1991).

(3) Similarly, the motor pattern of the two jaw adductors corresponded closely to the gape cycle. The jaw depressor showed low-level activity during slow tongue protraction, which tended to increase in intensity immediately prior to its offset at the onset of tongue projection. Though no antagonistic activity was seen in the two jaw adductors, the jaws did not open at this stage. It is possible that jaw adductors other than the two examined in this study play a role during this time. Rapid jaw opening during tongue retraction is accompanied by depressor activity.

We are grateful to Kenya's Office of the President and the Department of Wildlife and Conservation for permission to pursue research on Kenyan chameleons. Drs Gabriel Mutungi, Alex Duff-MacKay and Divindra Magon and the University of Nairobi provided facilities and other logistical support in Kenya. We thank S. Reilly for introducing us to problems in chameleon biology. Drs S. Reilly and B. Jayne were invaluable field partners. For laboratory assistance we thank R. Hirsch and J. So. D. Kraklau expertly prepared Fig. 1B. G. Lauder, S. Reilly, B. Jayne and two anonymous reviewers offered critical comments on the manuscript. This research was funded by NSF grants DIR 8820664 to G. Lauder, A. Bennett and R. Josephson and DCB 8812028 to A. Bennett.

References

- ALTEVOGT, R. AND ALTEVOGT, R. (1954). Studien zur Kinematik der Chamaleonzunge. *Z. vergl. Physiol.* **36**, 66–77.
- BELL, D. A. (1989). Functional anatomy of the chameleon tongue. *Zool. Jb. Anat.* **119**, 313–336.
- BELL, D. A. (1990). Kinematics of prey capture in the chameleon. *Zool. Jb. Physiol.* **94**, 247–260.
- BELS, V. L. AND BALTUS, I. (1987). First analysis of feeding sequences of *Chameleo dilepis*. In *Proc. 4th Ord. Gen. Meet. Soc. Europ. Herpet.* (ed. J. J. van Gelder, H. Strijbosch and P. J. M. Bergers), pp. 67–70. Nijmegen: Faculty of Sciences Nijmegen.
- BRAMBLE, D. M. AND WAKE, D. B. (1985). Feeding mechanisms of lower tetrapods. In

- Functional Vertebrate Morphology* (ed. M. Hildebrand, D. M. Bramble, K. F. Liem and D. B. Wake), pp. 230–261. Cambridge: Cambridge University Press.
- BRÜCKE, E. (1852). Über die Zunge der Chameleonen. *Sitz. Ber. Math.-Nat. Kl. Akad. Wiss. Wien* **8**, 62–70.
- DEWEVRE, L. S. (1895). Le mécanisme de la projection de la langue chez le caméléon. *J. Anat. Physiol.* **31**, 345–360.
- DUVERNOY, G. L. (1836). Sur les mouvements de la langue du caméléon. *C. R. hebd. Séanc. Acad. Sci. Paris* **2**, 349–351.
- ESTES, R., DE QUEIROZ, K. AND GAUTHIER, J. A. (1988). Phylogenetic relationships within Squamata. In *Phylogenetic Relationships of the Lizard Families* (ed. R. Estes and G. Pregill), pp. 119–282. Stanford: Stanford University Press.
- GANS, C. (1967). The chameleon. *Nat. Hist.* **76**, 52–59.
- GNANAMUTHU, C. P. (1930). The anatomy and mechanism of the tongue of *Chamaeleo carcaratus* (Merrem). *Proc. zool. Soc., Lond.* **31**, 467–486.
- GNANAMUTHU, C. P. (1937). Comparative study of the hyoid and tongue of some typical genera of reptiles. *Proc. zool. Soc., Lond.* **107B**, 1–63.
- HOUSTON, J. (1828). On the structure and mechanism of the chameleon tongue. *Trans. R. Irish Acad.* **15**, 177–201.
- KATHARINER, L. (1894). Anatomie und Mechanismus der Zunge der Vermiliger. *Jena, Z. Naturwiss.* **29**, 247–270.
- MIVART, S. G. (1870). On the myology of *Chamaeleo parsoni*. *Proc. Sci. Meet. Zool. Soc. Lond.* **57**, 850–890.
- RICE, M. J. (1973). Supercontracting striated muscle in a vertebrate. *Nature* **243**, 238–240.
- RIEPEL, O. (1981). The skull and jaw adductor musculature in chameleons. *Rev. suisse Zool.* **88**, 433–445.
- SCHWENK, K. AND BELL, D. A. (1988). A cryptic intermediate in the evolution of chameleon tongue projection. *Experientia* **44**, 697–700.
- SMITH, K. K. (1988). Form and function of the tongue in agamid lizards with comments on its phylogenetic significance. *J. Morph.* **196**, 157–171.
- SOKAL, R. R. AND ROHLF, F. J. (1981). *Biometry*. San Francisco: Freeman.
- TANNER, W. W. AND AVERY, D. F. (1982). Buccal floor of reptiles, a summary. *Great Basin Nat.* **42**, 273–349.
- WAINWRIGHT, P. C. AND BENNETT, A. F. (1992). The mechanism of tongue projection in chameleons. II. Role of shape change in a muscular hydrostat. *J. exp. Biol.* **168**, 23–40.
- WAINWRIGHT, P. C., KRAKLAU, D. M. AND BENNETT, A. F. (1991). Kinematics of prey capture in *Chamaeleo jacksonii*. *J. exp. Biol.* **159**, 109–133.
- ZOOND, A. (1933). The mechanism of projection of the chameleon's tongue. *J. exp. Biol.* **10**, 174–185.