

## TONGUE FUNCTION IN THE SALAMANDER *BOLITOGLOSSA OCCIDENTALIS*

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**Summary**—Many plethodontid salamanders feed by means of tongue projection. Aspects of this mechanism were studied in the species *Bolitoglossa occidentalis* under laboratory conditions. *Drosophila* were presented at varying controlled distances from the animal. The force and duration of tongue contact with the prey, plus the EMG activity in the tongue protractor and tongue retractor muscles, were recorded. Both force and duration of contact decreased with increasing distance of tongue projection. Various measures of protractor muscle EMG activity were independent of projection distance. Protractor and retractor muscle activities were synchronous and continued throughout the projection–retraction cycle. The latency of a ‘tongue pad to retractor muscle’ reflex was greater than the shortest durations of tongue contact. Furthermore, the longest durations of contact were always associated with the greatest forces exerted by the tongue. In the light of these findings, a model is proposed in which projection–retraction is co-ordinated by properties of the peripheral structures rather than by those of the central nervous system.

### INTRODUCTION

Tongue projection as a method of capturing prey is commonly used by amphibians. Projection in many species of urodeles is highly developed and the cycle of projecting and retracting the tongue is extremely fast; preliminary photographic observations on one species have suggested that it is complete in 50 ms or less (R. E. Lombard and D. B. Wake, unpublished).

The assumption which this study set out to test was that tongue projection–retraction is governed by a negative feedback control system analogous to those said to operate on oral structures in mammalian feeding (Sherrington, 1917; Jerge, 1964; Thexton, 1973, 1974), i.e. that tongue retraction is reflexly elicited by tongue contact with the prey.

### METHOD

The experimental subjects were eight adult *Bolitoglossa occidentalis*, collected in Guatemala. This species, a member of the family Plethodontidae, the lungless salamanders, was selected for experimental use because it feeds readily under laboratory conditions. All tropical plethodontids share a specialized tongue projection mechanism for obtaining prey (Wake, 1966). Detailed descriptions of the tongue are available (Tanner, 1952; Lombard and Wake, 1976) and only relevant elements are considered here. During feeding, the tongue is extended as a projectile from the mouth to the prey. This projectile consists of the tongue pad trailed by an elongate bundle of folded cartilages, retractor muscles, nerves and vessels enveloped in a mucosal sheath. The apparatus is protracted by means of a pair of complex subarcualis rectus muscles which extend posteriorly from their origin on the ceratohyal cartilages in the floor of the mouth to wrap around the paired, elongated and

slightly tapered epibranchial cartilages. The cartilages extend from the gular region posterodorsally over the insertion of the fore-limb (Fig. 1A). The subarcualis rectus muscle is spirally wound around the cartilage to form a bulb; upon contraction, the slightly tapered epibranchial of each side is forced anteriorly, out of the cavity of the respective muscle. At the same time, the entire muscle shortens and the free posterior end moves towards the gular region (Fig. 1B). The epibranchials conduct force by way of other cartilages (the paired first and second ceratobranchials) to the single midline basibranchial and thence to the tongue pad. The subarcualis rectus muscles are subsequently referred to in this paper as the ‘protractors’. The paired ‘retractor’ muscles (a combination of m. rectus cervicis profundus and m. rectus abdominis profundus) originate on the pelvis (posteroventral margin of the ischium) and pass without interruption to their insertion in the tongue pad. Immediately anterior to the heart at about the level of the gular fold, the muscles are lax and lie in one or two small loops. In contrast, the protractor muscles are slightly stretched when the tongue is at rest in the mouth.

The animals used were 37–43 mm long (snout-vent) and weighed 1.1 to 1.6 g. In the experimental situation the animals were housed in a horizontal clear plastic tube (2 cm dia) the floor of which was lined with damp paper. *Drosophila* stuck on to a metallized target with glycerin–albumen were presented to the animal. The target was mounted on a strain gauge as, for some experiments, was the tube housing the animal; the amplified strain gauge outputs gave a measure of force exerted by the tongue. The body contact of the animal with the wet paper constituted an indifferent electrode. This was employed in two ways: First, amplification of the signal at this electrode with reference to earth produced a 50 Hz sine

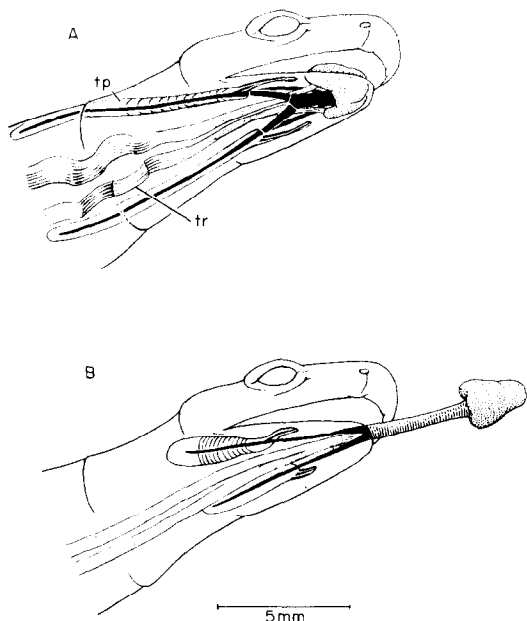


Fig. 1. A diagrammatic representation of the tongue projection mechanism in the salamander. In A, the tongue is shown partly retracted, and in B in projection. tr = retractor muscle of the tongue, tp = protractor muscle of the tongue. For full anatomical details see Lombard and Wake (1976).

wave (main interference) which disappeared when the animal's tongue was in contact with the earthed metallized target. Duration of contact could thus be measured. Second, the indifferent electrode was used when EMG activity was recorded using monopolar electrodes made from fine ( $25\ \mu\text{m}$  dia) wire insulated except for a 1–2 mm tip bent back as a hook. The electrodes were inserted, under cold anaesthesia, through the skin over the muscle. Typically the electrode came to lie in close contact with the muscle in the lymph space, but occasionally the electrode penetrated the muscle. The technique was dictated by the extremely small size of the animals and the mobility of the muscles examined. Electrodes were implanted near *m. subarcualis rectus I* and, in some experiments, in *m. interhyoideus* (a superficial, transverse constrictor of the gular region), *m. adductor mandibulae*, *m. rectus cervicis superficialis*, *m. rectus cervicis profundus* and *m. rectus abdominis profundus*. Electromyographic (EMG) activity was preamplified by battery powered amplifiers, the output of which was transformer isolated from earthed circuits. The amplifier band pass (200–5 kHz) was selected so as to discriminate against EMG signals arising more than a millimeter or so from the electrode tip (Håkansson, 1957; Hayes, 1970; Trimble, Zuber and Trimble, 1973). EMG activity was quantitated by integration (Bouisset and Goubel, 1973). In some experiments, a beam of light was aligned at right angles to the trajectory of the tongue and so as to fall on a light-sensitive device. The changes in light intensity as the tongue entered the beam were sufficient to produce signals which, when amplified, allowed timings of tongue movement towards the target to be made. Strain gauge, light device and

either target contact or EMG signals were recorded on a storage oscilloscope and on magnetic tape. At each feeding sequence, the distance of the snout from the target was estimated, viewing both against a background of  $1\ \text{mm}^2$  graph paper; light device to target distance was also measured.

Some animals were anaesthetized by immersion in solutions of chlorbutanol (0.2 per cent), urethane (2.5 per cent) or methohexitone sodium (1 per cent). The tongue was then pulled out to its full length, placed on stimulating electrodes and reflex EMG activity was recorded in the retractor muscle in response to electrical stimulation of the tongue.

Other animals were pithed and either the tip of the tongue or the muscle at the posterior tip of the epibranchial was attached to an isometric strain gauge. Direct stimulation of the muscle enabled twitch contractions of the muscle to be recorded.

## RESULTS

While under experimental conditions, an animal would eat as many as 29 flies at one sitting. The consumption of flies was high at laboratory temperatures over  $21^\circ\text{C}$  and low when the temperatures were below  $18^\circ\text{C}$ ; most animals either did not feed below  $18^\circ\text{C}$  temperature or, if they did, the tongue projections were short and erratic as judged by successive measurements of force or duration of contact with prey. At  $17.8^\circ\text{C}$ , 42 trials had a mean projection length of 10.3 mm (range 6–15 mm,  $s = 2.89$ ); at  $25.6^\circ\text{C}$ , 28 trials had a mean projection of 15.9 mm (range 12–19 mm,  $s = 1.75$ ). Animals successfully captured prey placed at distances of 2 to 19 mm. The maximal distance the tongue was projected from the mouth is equivalent to 44 per cent of body length of the largest animal. There was no perceptible variation in distances of projection as a function of body size. At distances greater than 17 mm, the animals frequently took no notice of the prey and, at distances less than 5 mm, usually backed away, thus increasing the distance to the target. During the course of observing about 200 tongue projections, only on a few occasions did the tongue fail to reach the target and only on 15 or so occasions did the tongue hit the target and yet fail to return the fly to the mouth.

Approximate figures for tongue velocity (in the range 3–5 m/sec) were periodically obtained by measuring the time elapsed between the tongue crossing a light beam and it reaching the target. In one experiment at  $25.4^\circ\text{C}$ , the light-beam-to-target distance was varied from 5–11 mm by moving the target, but the animal-to-light-beam distance was kept as constant as possible at about 4 mm. Time elapsed was then plotted against distance; the calculated slope of the graph gave a value of 3.4 m/sec which was consistent with previous spot values. This figure is an average for the terminal velocity as the tongue has initially to accelerate from rest. However, as the plot appeared to be linear ( $r = 0.77$ ), the implication is that acceleration of the tongue occurs largely before crossing the light beam about 4 mm from the snout.

When both the target and the animal housing were mounted on strain gauges, the reaction on the body during a tongue projection coincided in time with the onset of force exerted by the tongue on the target,

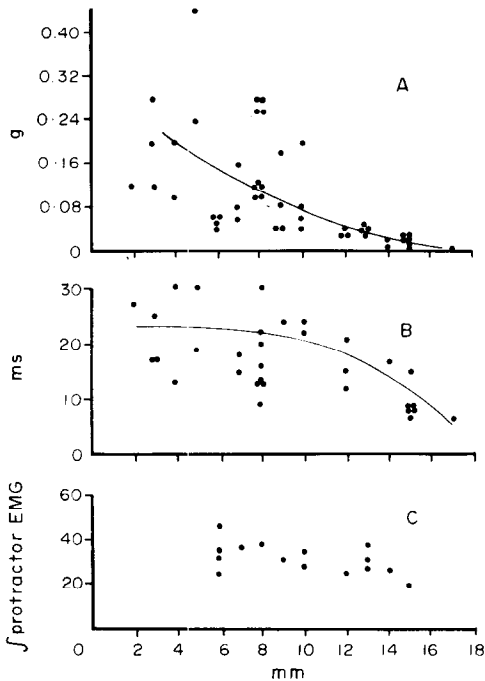


Fig. 2. A. Graph of the relationship between the force exerted on prey (vertical axis—g) and tongue projection distance (horizontal axis—mm). The force exerted by the tongue was measured at peak value. There is a general, and apparently non-linear, decrease of force exerted with increasing distance to prey. Pooled data from 3 experiments carried out at 21–25°C. As a guide, a line has been drawn by eye so that it passes through the mean ( $\pm$  1 S.E.) value in each 2 mm 'bin' on the abscissa. B. Graph of the relationship between duration of tongue contact with prey (vertical axis—ms) and tongue projection distance (horizontal axis—mm). With increase of projection distance, there is a general decrease of contact time, more marked at distances greater than 10 mm. As a guide, a line has been drawn as in A. C. Graph of the relationship between integrated protractor EMG activity (vertical axis—arbitrary units) and tongue projection distance (horizontal axis—mm). All data points were obtained in the same animal in one experimental session. In this case, there is not only no indication of increased EMG activity with increased projection distance, but, if anything, the reverse.

i.e. the force producing acceleration of the tongue was not measurable. Tongue projection is therefore not a simple ballistic event; force is usually exerted for a significant time after the instant of contact with the prey.

Force of impact on target decreased with increased distance to target (Fig. 2A). Mean force in 20 trials at distances of 11 to 17 mm was 0.026 g (range 0.0–0.04 g). In this series of trials, projection distances of 6 to 10 mm showed a great range of force of impact (mean for 24 trials 0.12 g; range 0.04 to 0.28 g). At distances of 5 mm or less, force of impact on target was significantly greater than at longer distances (mean force for 8 trials 0.21 g; range 0.1 to 0.44 g).

The length of time the tongue remained in contact with the target also decreased with increased distance of tongue projection, particularly at distances greater than 10 mm (Fig. 2B). This is especially noted in respect to maximal times of contact but minimal con-

tact times were recorded over a range of projection distances.

EMG activity recorded from the protractor and retractor muscles tended to be synchronous (Fig. 3A, B, C). The patterns of activity were of three types. 1. A single synchronous burst of EMG activity with sudden onset and large amplitude (200  $\mu$ v) in both protractor and retractor muscles (Fig. 3A), lasting 60–90 ms. 2. A single synchronous burst of EMG activity similar to the foregoing but preceded by a lower amplitude (40  $\mu$ v) period of EMG activity with a duration of 150 ms or more (Fig. 3B). 3. EMG activity starting as in either 1 or 2 but continuing into a series of moderate amplitude, rhythmic bursts of about 40 ms duration and about 200 ms period (Figs. 3C, D).

Types 1 and 2 were associated with tongue projections in which no food was returned to the mouth.

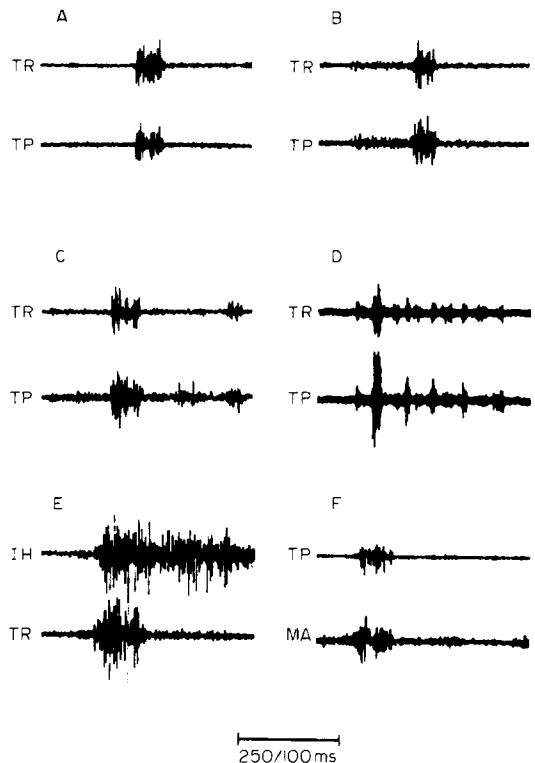


Fig. 3. Examples from different animals of EMG activity during tongue projection. The time calibration for record D is 1000 ms and for all other records, 250 ms. The same gains have been used throughout, and the EMGs have been coded as follows: TP—tongue protractor muscle; TR—tongue retractor muscle; MA—adductor muscle of the mandible; IH—interhyoideus muscle. In record A (tongue projection distance 10 mm), no prey was returned to the mouth. Records B and C were obtained in the same animal and show a tongue projection (> 10 mm) where the prey was first missed (B) and then captured successfully (C). Record D shows a successful prey capture and subsequent rhythmic activity. Record E shows an unusually long duration burst of activity in the retractor muscle coupled with prolonged activity in the interhyoideus. This record demonstrates the degree of discrimination in 'pick up' from electrodes separated by only a few millimetres. Record F shows the activity in the tongue protractor and mandibular adductor muscles.

Type 2 activity (with 'preparatory activity') was seen when prey was at a distance greater than 10 mm. Type 1 activity was uncommon and was seen in association with short projection distances (<10 mm). Type 3 activity was associated with the capture of prey and with subsequent cyclic oral movements. A few recordings were made from electrodes in or on *m. interhyoideus* and *m. adductor mandibulae* (Figs. 3E, F) and activity was recorded during tongue projection which was synchronous with the activity in the protractor muscle. Although most EMG activity was synchronous (non-reciprocal), activity could be recorded which appeared in one muscle but not in another only a few millimeters away (Fig. 3E); consequently the question of the synchronous activity being an artifact due to overlapping recording fields or to cross talk between signal channels could be excluded. Similar non-reciprocal EMG activity in antagonistic muscles has been described in the limbs of the salamander during locomotion (Szekely, Czeh and Voros, 1969). Furthermore, although the retractor electrode was situated very close to the heart, no trace of an ECG was recorded and, although the protractor electrode was inserted very close to the *m. levator scapulae* and *m. dorsalis trunci*, activity was not recorded during movement of the ipsilateral fore-limb alone. The time, measured from onset of protractor EMG activity to contact with the target, varied from 8–19 ms; recorded times greater than 10 ms were always associated with an EMG commencing as in Type 2 activity ( $\geq 10$  per cent of total observations) where it was difficult to define the onset of the main burst.

Recordings made from the abdominal portion of the retractor muscle failed to show signals characteristic of propagated muscle action potentials in the proximity of the electrode during tongue projection, although reflex EMG activity could be elicited at the site, by pinching the tail. At very high gain ( $5 \mu\text{V}/\text{cm}$ ), activity could be detected that sometimes appeared

either as a low amplitude filtered version of the signal in the cervical part of the muscle or as a quite different monophasic low amplitude and low frequency signal starting just after the main burst of activity occurred in the cervical part. Synchronization of EMG activity with that of the protractor muscle was seen both in ipsilateral and contralateral recordings from the retractor, interhyoid and mandibular adductor musculature. Despite the similar appearance of the signals in the protractor and retractor muscles, when the full wave rectified signals were integrated, they were neither equal nor in constant proportion; for example, the size of integrated retractor EMG varied by up to 30 per cent as against standardized values for protractor EMG activity. Furthermore, the signals so quantitated bore no consistent relationship to the distance the tongue projected (Fig. 2C). Peak-to-peak amplitude and duration also appeared to have no relationship to projection distance.

Some general characteristics of the musculature were explored by direct stimulation in the pithed animal. Due to the extreme fragility of the muscles, attempts at obtaining length-tension curves failed. Isometric twitch-times were, however, established for the protractor and retractor muscles. Both muscles had a time-to-peak contraction of 25–30 ms and a half-relaxation time of the same order.

The latency of a tongue-withdrawal reflex was measured by electrically stimulating the passively extended tongue 'stalk' and recording EMG activity in the cervical part of the retractor muscle. Under chlorobutanol anaesthesia, no reflex was elicited and, under urethane anaesthesia, it was only elicited, with a latency of about 100 ms, just before recovery. However, under the influence of methohexitone, which could be expected as a barbiturate derivative to spare short latency reflex activity in amphibians (Richens, 1969), it was elicited with successively shortening latency as anaesthesia lightened. The minimum value obtained under light methohexitone anaesthesia was 12 ms (Fig. 4).

## DISCUSSION

The assumption made at the outset was that sensory input controls the course of tongue projection-retraction and in particular that some negative feedback system operates during tongue contact with prey, initiating retraction. However, many results obtained were not consistent with this view. On theoretical grounds either absence or suppression of reflex control mechanisms would be expected under conditions where velocity of action is the prime requisite; feedback systems introduce delays not present when activity is preprogrammed (Denier van der Gon and Wieneke, 1969).

Although visual cues were apparently used to modify some components of the pattern of EMG activity, to the extent that a preparatory burst preceded the main activity when the prey was distant, tongue projection was otherwise the result of a rather standardised pattern of EMG activity. In this pattern, both protractor and retractor muscles showed only synchronous activity, the magnitude of which did not relate to projection distance. As the time from the onset of the main (projection) burst of EMG activity

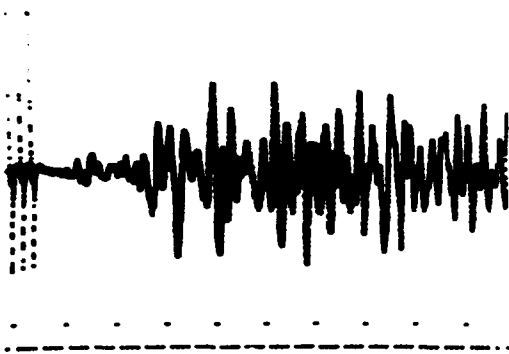


Fig. 4. Record of EMG activity in the cervical part of the tongue retractor muscle following electrical stimulation of the tongue. Under methohexitone anaesthesia, the tongue was pulled out to its full extent and the tip placed on the stimulating electrodes; three 10 mA stimuli, 2 ms wide at 2 ms intervals elicited the shortest latency reflex. Prior to stimulation, there was no sign of EMG activity. The lower (time) trace shows 10 ms intervals. Although the earliest sign of EMG activity occurs 12 ms from the onset of stimulation, high amplitude activity does not occur until much later.

to the contact with the target was in the range 8–19 ms, the major part of the protractor activity (usually lasting 65 ms) occurred well after contact and this EMG activity did not show the expected signs of modulation due to sensory feedback. The isometric twitch of the protractor muscles had a time to peak of 30 ms and a half-relaxation time of the same order, so it is clear that only a fraction of the total mechanical activity was expressed in actual projection or acceleration of the tongue out of the mouth. Even at close range with the tongue staying on the target a relatively long time, say 30 ms, protractor EMG activity (approx 65 ms duration) extended into the retraction phase; mechanical expression of the EMG activity would consequently extend even further into that phase and presumably into the subsequent intra-oral phase when the hyobranchial skeleton had returned to its original position. This does not fit the usual pattern of a negative feedback control system. Furthermore, the heaviest tongue contact forces were, on average, associated with the longest contact times whilst the lightest contact forces were associated with the shortest contact times. Although measures of force and duration of tongue contact varied apparently independently of each other in individual tongue projections, both average force and average duration of contact decreased with projection distance. Such observations are quite inconsistent with accurate reflex control, particularly when associated with the finding that the shortest latency (experimentally elicited) tongue retractor reflex was longer than many of the naturally occurring durations of tongue contact. Furthermore, the change in contact duration with tongue projection distance (e.g. 9 ms at 15 mm and 22 ms at less than 5 mm) cannot be explained simply as a difference in 'travelling time', i.e. due to the tongue taking longer to reach the more distant prey. Because the tongue velocity was about 3.4 m/sec, it would take only 3 ms longer to reach the more distant prey, leaving 10 ms loss of contact time unaccounted for at the greater distance.

Our findings are easily explained if it can be accepted that the co-ordination of tongue projection–retraction may be less dependent upon sensory feedback and precise central nervous control than it is upon the characteristics of the peripheral structures. The starting point for this hypothesis is the observation that, at rest, the protractor muscle is slightly stretched, due to tension in the unfolded hyobranchial skeleton, whilst the retractor muscle is slack, even folded (Lombard and Wake, 1976). In the extended tongue, the reverse exists. The data we obtained suggest the following model:

1. Following detection of prey within certain ranges, both protractor and retractor muscles are simultaneously activated. Although this may be a pre-programmed act, the activity does not necessarily have to be accurately metered.

2. The tongue projects because the protractor muscle is at or near its optimum length for force production whilst the retractor is not.

3. As the tongue projects well outside the mouth, the retractor muscle is stretched to an optimum length so that activation of the retractor muscle, as demonstrated by EMG, reaches its maximum mechanical expression.

4. At any distance from the snout, the amount of force available on the target is a function of the opposing length–tension curves of the protractor and retractor muscles. Similarly, duration of contact depends upon the time taken for the retractor to take up the slack existing in this muscle. At the greatest distances of tongue projection, this slack has been completely taken up by the act of projection so that the force and duration of contact approaches zero.

5. At some point on the projection path, the retractor muscle becomes more powerful than the protractor, due to the respective length–tension relationships and/or additional non-linear characteristics of the muscles (Joyce, Rack and Westbury, 1969). Retraction therefore follows.

6. Retraction occurs against a progressive deceleration as the protractor is still firing. Consequently, once the hyobranchial skeleton has been returned to its original position, rebound might result from continued activity in the protractor muscle. However, assuming that the mouth is by then closed, this could simply result in pressing the prey against the vomerine teeth in the palate, thus initiating processing of the prey.

According to this model, co-ordination of feeding in the salamander is more a function of the properties of peripheral structures than central nervous organisation. This conclusion, in our view, is preferable to others based on peripheral feedback mechanisms or precise pre-programming, but does not of itself demand total exclusion of other mechanisms.

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