How Does the Toad Flip Its Tongue? Test of Two Hypotheses

Abstract. *Two conflicting hypotheses purport to explain the mechanism generating the lingual flip in frogs. The first suggests that the intrinsic tongue muscles are stiffened, rotate over the symphysis and catapult the soft tissues; the second suggests that the hyoid suddenly moves forward and transfers its momentum to propel the tongue. High-speed cinematography and synchronized electromyography show that the tongue is rotated over the symphysis by a complex of rods formed from stiffened intrinsic tongue muscles. As the flip occurs even when the hyoid is immobilized, the hyoid momentum hypothesis does not apply. The tongue is then propelled by sets of fibers locked into connective tissues. With activation, these become rigid rods that form a muscular ballista.*

In most frogs the tongue lacks an intrinsic skeleton. Its soft tissues attach near the mandibular symphysis rather than along the buccal floor and can be rotated out of the mouth, so that the fleshy tip (which is dorsal and posterior at rest) impacts downward on the prey. The propulsive mechanism remains in question. Two drastically conflicting hypotheses have been proposed, and both depend on different actions of the muscles interconnecting the tongue, the mandibular symphysis, and the hyoid plate, as well as the hyoid to the shoulder girdle (Fig. 1).

The first or ballista hypothesis is based on the assumption that propulsion is due to the musculature connecting tongue to symphysis (genioglossus) and to muscles (submentalis) that bridge the mandibles near the symphysis (*1, 2*). In the most recent version of this view (*3–5*) it is proposed that lingual protrusion depends on stiffening of these two muscle masses, to form a lever system that flips the fleshy tip toward the prey as a medieval ballista or trebuchet flipped a boulder, or as one flips the strings on a mop.

In the second or hyoid hypothesis (*6, 7*) it is proposed that the hyoid is first retracted (or stabilized) by the sternohyoideus muscle and that the geniohyoideus muscle in the floor of the mouth is tensed in opening the mouth (prior to the flip). Once the sternohyoid stops firing, the hyoid is accelerated anteriorly, communicates its stored energy to the tongue, and indirectly flips the soft lingual tissues over the symphysis. While the lingual muscles are stated to have some role in pulling the mass of the tongue toward the symphysis, most of the energy for the flip is supposedly generated by the geniohyoideus.

The two hypotheses also differ with respect to interpretation of the retraction of the tongue. In the first (ballista) hypothesis, it is assumed that the hyoid may move, but that lingual retraction is primarily driven by the hyoglossal muscle. In the second (hyoid) hypothesis, the intrinsic elastic recoil of the hyoid supposedly returns it immediately after the tongue contacts the prey; the hyoglossus is assumed to act later, after the hyoid has returned to its original position.

The families of anurans show a great diversity of arrangements of the tongue and its muscles (*2, 5*). However, the two hypotheses apply to the conditions in the genera *Rana* (Ranidae) and *Bufo* (Bufonidae), even though the tongue morphologies show some differences. We have reanalyzed this system, as part of a description of the tongue-protrusion mechanism in the toad *Bufo marinus*, and have concluded that the ballista hypothesis explains the observations and that the hyoid hypothesis may be falsified (*8*). In addition to dissecting the tissue and recording multiple prey-capture events by high-speed cinematography, we stimulated all the relevant muscles individually and in combinations to obtain an indication of their mechanical capacities. While making the high-speed films, we also recorded electromyograms from all muscles during the flip and determined their actual activation sequences during tongue protrusion and retrusion (*9*). Finally, we achieved several serendipitous and planned manipulations of the system.

Figure 2 shows tracings from an actual flip sequence. For each of six major muscles it also shows the predictions of the two hypotheses and the mean muscular activities as determined from electromyograms of a series of flips (*10*). The electromyograms are in good agreement with the ballista hypothesis and provide the first basis for rejecting the hyoid one. Thus, (i) the sternohyoid is only mildly (20 percent of maximum) active prior to the flip (Fig. 2a), continues at a very low level during the flip (Fig. 2, b to d), but becomes more active during tongue retraction (Fig. 2, e to f). (ii) The muscles connecting the symphysial tips and the tongue (genioglossus and submentalis) show peak, rather than minor, activities just as the tongue is flipped (Fig. 2, b and c), but insignificant action after retraction (Fig. 2, e to g). (iii) The hyoglossus reaches its maximum immediately, as soon as the tongue starts to retract (Fig. 2e); its action decreases thereafter, exactly opposite to the prediction of the hyoid hypothesis.

The second point of falsification of the hyoid hypothesis relates to its demand that the protruded tongue be flaccid. However, films of tongue flips which missed the prey (because the animals were misdirected to flip at mirrors) disclose that the genioglossus in the everted tongue is in fact rigid and remains directed at the prey; the tongue does not continue to flip around the lower jaw toward the skin of the throat as if it were flaccid. During retraction, the lingual tip dimples and there is a deformation of its distal periphery, rather than retraction of

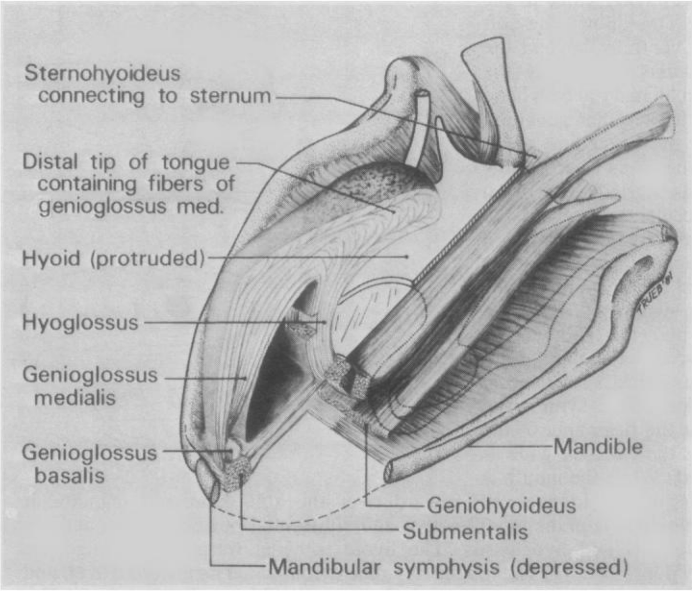

Fig. 1. Cross-sectional view of the tongue of an adult *Bufo marinus* at the beginning of a flip. Note the depression of the symphysis and the position of the geniohyoid and sternohyoid muscles, which can shift the hyoid by relative contraction. The ballista hypothesis indicates that the submentalis and the genioglossi become rigid so that the rod-shaped genioglossus medialis can lift the soft tissues of the tongue toward and over the mandibular symphysis. In the hyoid hypothesis, it is assumed that the momentum is imparted to the tongue by the hyoid plate, here shown in its protruded position. The tongue then continues from this position (just before that in Fig. 2b), rotates over the mandibular symphysis, and the momentum of the soft tissues impacts them onto the prey.

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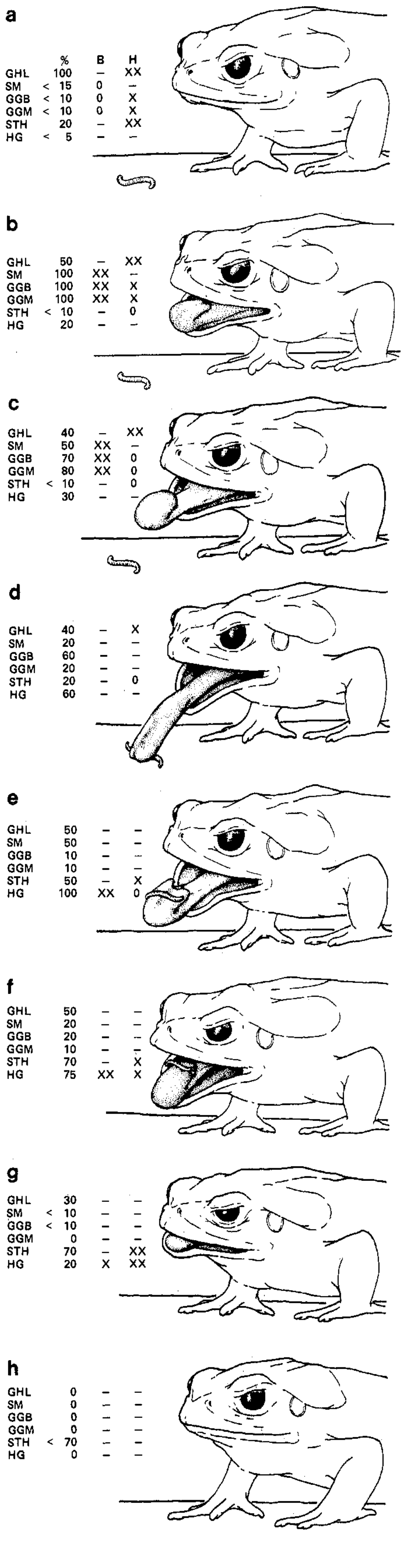

a

		%	B	H
GHL		100	–	XX
SM	<	15	0	–
GGB	<	10	0	X
GGM	<	10	0	X
STH		20	–	XX
HG	<	5	–	–

b

		%	B	H
GHL		50	–	XX
SM		100	XX	–
GGB		100	XX	X
GGM		100	XX	X
STH	<	10	–	0
HG		20	–	–

c

		%	B	H
GHL		40	–	XX
SM		50	XX	–
GGB		70	XX	0
GGM		80	XX	0
STH	<	10	–	0
HG		30	–	–

d

		%	B	H
GHL		40	–	X
SM		20	–	–
GGB		60	–	–
GGM		20	–	–
STH		20	–	0
HG		60	–	–

e

		%	B	H
GHL		50	–	–
SM		50	–	–
GGB		10	–	–
GGM		10	–	–
STH		50	–	X
HG		100	XX	0

f

		%	B	H
GHL		50	–	–
SM		20	–	–
GGB		20	–	–
GGM		10	–	–
STH		70	–	X
HG		75	XX	X

g

		%	B	H
GHL		30	–	–
SM	<	10	–	–
GGB	<	10	–	–
GGM		0	–	–
STH		70	–	XX
HG		20	X	XX

h

		%	B	H
GHL		0	–	–
SM		0	–	–
GGB		0	–	–
GGM		0	–	–
STH	<	70	–	–
HG		0	–	–

Fig. 2. Tongue protrusion and prey capture by adult *Bufo marinus*. The illustrations represent tracings from sequences filmed at 400 frames per second [the electromyogram (EMG) values represent means from at least ten sequences involving multiple toads (*9, 10*)]. The abbreviations on the left side of each figure refer, respectively, to the geniohyoideus lateralis (*GHL*), submentalis (*SM*), genioglossus basalis (*GGB*), genioglossus medialis (*GGM*), sternohyoideus (*STH*), and hyoglossus (*HG*). The values following upon each refer to the percentage of maximum activity observed in the muscles during 6 to 12 flips (represented as spike number times mean spike amplitude for 7-msec intervals) at positions similar to those shown. The sequence from open to closed mouth takes approximately 145 msec. Adjacent to the EMG values are code symbols indicating the values predicted by the ballista (*B*, column 2) and hyoid (*H*, column 3) hypotheses (–, no prediction; 0, no activity; X, low activity; XX, high activity). (a) Toad in anticipatory position with body rotated over forelimbs. The mouth is still closed but the lateral geniohyoid is highly active relating to protrusion of hyoid and facilitating ventrad rotation of the mandibular tips as soon as the mouth opens (next frame). (b) The mouth has opened and the base of the tongue has lifted toward the symphysis. The soft tissues of the lingual pad trail the stiffened base which is already rotating over the depressed mandibular symphysis. Throughout this phase, the submentalis, genioglossus basalis, and genioglossus medialis are highly active, while the activity in the geniohyoideus lateralis and sternohyoideus have dropped to about one-half of their previous values. (c) The base of the tongue has now rotated 180 degrees from its resting position to point toward the prey, extending over and beyond the depressed symphysis. The soft tissues of the lingual pad are still trailing the tip, but are being catapulted toward the prey. Activity in all lingual muscles has dropped from the previous values, indicating that the peak of energy input for propulsion has passed. (d) The soft tissues of the lingual pad have now reached their full extent and contacted the prey. (The sequence illustrated shows the initial position of the prey fairly close to the mandibular symphysis. This allows the soft distal portion to rotate ventrally beyond the stiffened lingual base, thus delineating the distal tip of the extended lingual rod by a bend in the tongue.) Muscle activity has generally dropped from that in the preceding view, except for that in the hyoglossus where it now rises. (e) Retraction has started. The distal soft tissues are first accelerated toward the mouth, apparently by the hyoglossus which now shows maximal activity, so that they pass the stiffened lingual base which remains beyond the mandibular symphysis. Both the genioglossus lateralis and the submentalis have increased activity, as have the sternohyoideus and hyoglossus. This is reflected in the secondary depression of the symphysial region, which occurs at the beginning of the retraction and increases the effective gape as tongue and prey enter. (f) While the mouth is already closing, the tip of the tongue rolls inward over the still depressed mandibular symphysis. The geniohyoideus lateralis is still active and substantial but decreasing activity occurs in the sternohyoideus and hyoglossus. The hyoid is being retracted although the symphysis is still slightly depressed. (g) The tongue has almost completed its inward travel, and the hyoglossal activity has dropped sharply, although the sternohyoid remains active. (h) The toad has now closed its mouth and the geniohyoideus lateralis and the intrinsic lingual muscles are silent. However, the sternohyoid continues to be active, as are some of the more posterior muscles (not shown) which will be involved in swallowing and, of course, ventilation.

the entire tongue; also the stiffened genioglossal rod remains beyond the symphysis while the soft tissues have already retracted. These observations suggest that retraction is controlled by specific fibers of the hyoglossus rather than by elastic recoil of the hyoid plate.

The critical aspect of the hyoid hypothesis is that momentum is imparted by forward movement of the hyoid. To test whether the observed movement was necessary for lingual propulsion, we solidly wired the hyoid plate to the junction between xyphoid process and the sternum (in two toads). This operation prevented hyoid protraction, but the animals still fed normally. The hyoid momentum hypothesis is then falsified.

Apparently, the tongue of *Bufo* is flipped by a ballista mechanism. The long fibers of the genioglossus medialis form a rod along the surface of the tongue, and the short fibers of the genioglossus basalis form a wedge deep to the anterior end of the rod. Activation of the submentalis depresses the symphysial tips, and this muscle swells upward pushing against the two parts of the genioglossus. The effect is to pull the anterior attachment of the genioglossal rod ventrally and to lift the more posterior portion of the rod (by the push of submentalis against genioglossus basalis). This amounts to a force couple that rotates the rod over the symphysis and flips the extensile tissues of the lingual tip. The stimulation experiment shows the stiffening, and the electromyograms confirm the sequence of activation. When an electrode lead accidentally anchored the genioglossus basalis into the underlying connective tissue, the tongue did not flip, even though most of the lingual mass remained free. Shift of the electrode, so that it was entirely in the muscle, allowed the toad to feed without difficulty, confirming the importance of the muscular elevators.

Why then does the hyoid move anteriorly in unoperated animals? Retraction of the tongue mainly involves the hyoglossus. As the tongue more than doubles its resting length, this muscle must be substantially stretched, yet it has to exert its greatest force at maximum stretch, just after impact on distant prey. Protraction of the hyoid plate is apparently advantageous because it places the extended muscle into an improved position on its length-tension curve.

In recording the ballista method of tongue flipping, our study describes an unusual pattern for lingual support and propulsion. In other animals with projectile tongues, such as salamanders, chameleons, and woodpeckers (*11*), the protrusible tongue is mounted on the hyoid, and hyoid movement everts it or positions it beyond the mouth for further propulsion. *Bufo* and all known anurans lack such an intrinsic lingual skeleton (*2*, *5*). Instead, there are sets of muscle fibers locked into a connective-tissue framework that limits their shortening. Stimulation transforms these sets into rigid rods that transmit forces at right angles to the lines of action of their component muscle fibers. Thus, it is the swelling of the muscle as much as its shortening that rotates the lingual frame and produces the ballista. While lateral transmission of muscular force appears unusual, it does occur in a number of other situations, an obvious example being the protrusible human tongue (*12*).

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9. High-speed cinematography (140, 200, and 400 frames per second) and synchronized electromyograms (EMG) of the lingual and hyoid muscles were recorded from 17 unanesthetized and unrestrained toads *(Bufo marinus)* fed on mealworms. Analysis included 36 feeding sequences with good simultaneous cine and EMG, 76 sequences with EMG only, and 100 films of control sequences. High-speed films were obtained with a Hycam 16-mm movie camera from which a signal from the shutter mechanism provided a frame marker that was stored on tape and permitted correlation of tongue position with muscular activity. After an intraperitoneal injection of tricaine methanesulfonate (0.04 mg per gram of body weight), bipolar fine-wire electrodes (0.076-mm Teflon-coated stainless steel; 1-mm bared tips; Medwire Inc.), inserted into 22-gauge hypodermic needles, were passed subcutaneously from the parotid region toward the buccal cavity and submucosally into the appropriate muscles. The wire was kinked several times during removal of the hypodermic needle. Reverse stimulation of the electrode was used to check electrode placement. Generally, four electrodes were implanted for each experiment and their external ends were soldered to a harness of earphone wire which, in turn, was sutured to the animal's back. One to two days after the electrodes were implanted, the animals were offered food in a photo cage with a 45° mirror that provided simultaneous frontal and lateral views of the head. Electromyogram signals were amplified through 26A2 Tektronix preamplifiers, Honeywell 117 DC amplifiers, and stored on a Honeywell 5600 1-inch tape recorder. Films projected from a Lafayette analytical projector were analyzed frame by frame, and the position of the tongue relative to the head was traced; EMG signals were processed and plotted with a minicomputer (J. Beach, G. C. Gorniak, C. Gans, *J. Biomech.*, in press).
10. The EMG magnitudes were keyed to the film frames, reflecting 5- or 7-msec intervals (150 or 200 frames per second). The delay between mechanical muscle activity and electrical stimulation in frogs at 20°C is also 5 msec [B. C. Abbott and A. J Brady, in *Physiology of the Amphibia*. J. A. Moore, Ed. (Academic Press, New York, 1964), vol. 1, p. 329].
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Tritiated Imipramine Binding Sites Are Decreased in the Frontal Cortex of Suicides

Abstract. *Binding characteristics of tritiated imipramine were determined in the frontal cortex of suicides and well-matched controls. Maximal binding was significantly lower in brains from the suicides. This finding is consistent with reports of decreased tritiated imipramine binding in the platelets of patients diagnosed as having a major affective disorder.*

Briley *et al.* (*1*) reported decreased binding of [^{3}H]imipramine in the platelets of patients suffering from clinically significant depression. The change in this binding characteristic appears to be related more to diagnostic category (affective disorder) than to clinical status or mood, since binding properties are not correlated with measures of depression and remain unaltered as a function of treatment outcome (*2*). A recent report (*3*) indicated that [^{3}H]imipramine binding is increased in platelets from patients with unipolar or bipolar disorders. However, the lack of specific information regarding the diagnostic criteria as well as the absence of binding data make evaluation of the report difficult. Binding sites for [^{3}H]imipramine have also been described in various regions of the brains of animals (*4*) and humans (*5*). Both receptor sites—brain and platelet—possess virtually identical binding characteristics, and it has been postulated that they may serve as useful biological substrates for advancing our understanding of affective disorders.

Evidence that [^{3}H]imipramine binding sites in both platelets and brain tissue are functionally related is provided by preliminary animal studies. Arbilla *et al.* (*6*) reported parallel decreases in [^{3}H]imipramine binding in cat platelets and brain tissue after long-term imipramine treatment. However, to determine whether the decrease in [^{3}H]imipramine binding observed in platelets from patients with affective disorders is an indicator of similar changes in the brain, it is necessary to examine binding in samples from deceased patients with a similar diagnosis. Because of the prevalence of affective disorders in people who commit suicide (*7*), we decided to determine the binding characteristics of [^{3}H]imipramine in the frontal cortex of suicide victims and matched controls.

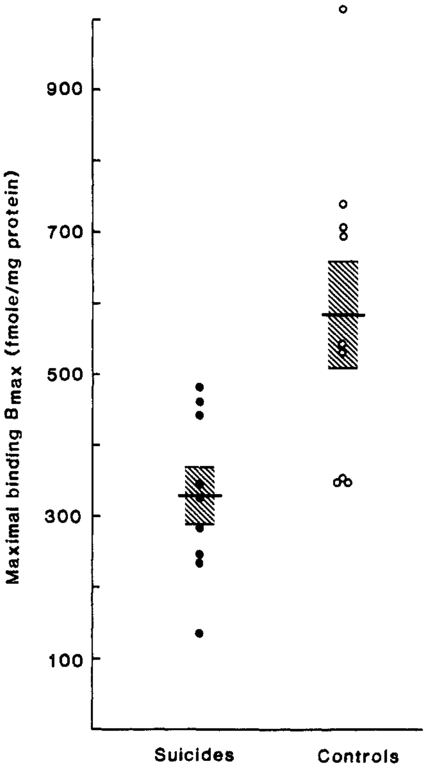

Fig. 1. Comparison of maximal binding of [^{3}H]imipramine in frontal cortex samples from suicide and control subjects. Each point represents an individual subject. Binding values were calculated by Scatchard analysis with six concentrations of [^{3}H]imipramine. Solid lines and hatched areas represent the means and standard errors, respectively, for each group. The difference in B_{max} values is statistically significant ($P < .01$).

Brain samples from nine suicide victims and nine controls were obtained at autopsy at the New York City Medical Examiner's Office (*8*). There were no significant differences between the two groups with respect to age, sex, and elapsed time between death and autopsy (Table 1). Frontal cortex samples corre-