

Specialized bat tongue is a hemodynamic nectar mop

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Nectarivorous birds and bats have evolved highly specialized tongues to gather nectar from flowers. Here, we show that a nectar-feeding bat, *Glossophaga soricina*, uses dynamic erectile papillae to collect nectar. In *G. soricina*, the tip of the tongue is covered with long filamentous papillae and resembles a brush or mop. During nectar feeding, blood vessels within the tongue tip become engorged with blood and the papillae become erect. Tumescence and papilla erection persist throughout tongue retraction, and nectar, trapped between the rows of erect papillae, is carried into the mouth. The tongue tip does not increase in overall volume as it elongates, suggesting that muscle contraction against the tongue's fixed volume (i.e., a muscular hydrostat) is primarily responsible for tip elongation, whereas papilla erection is a hydraulic process driven by blood flow. The hydraulic system is embedded within the muscular hydrostat, and, thus, intrinsic muscle contraction may simultaneously increase the length of the tongue and displace blood into the tip. The tongue of *G. soricina*, together with the tongues of nectar-feeding bees and hummingbirds, which also have dynamic surfaces, could serve as valuable models for developing miniature surgical robots that are both protrusible and have highly dynamic surface configurations.

biomechanics | fluid dynamics | lingual papillae | feeding kinematics | soft robots

Glossophagine bats and nectar-feeding birds hover in front of flowers and use their long tongues to collect nectar (Fig. 1A). Hovering is energetically expensive, and nectar resources are limited in the wild, so birds and mammals have developed specific strategies to gather nectar efficiently from flowers—one of which is to have a long, protrusible tongue. Hummingbird and bat tongues are so long that they are housed in an elongated bill or rostrum. During nectar feeding, these tongues typically elongate to more than double their resting lengths (1, 2).

In addition to having extraordinarily long and protrusible tongues, these animals also have elaborate structures on the tongue tip. Hummingbirds have bifurcated tubular tongue tips, formed by curled keratinous lamellae (3). During feeding, nectar is trapped within and between the tubular tongue tips and carried into the mouth (4). The tip of a nectar-feeding bat tongue is not tubular; instead, it is covered with many elongated, conical papillae. These hair-like papillae give the tongue tip a brush- or mop-like appearance (Fig. 1B). For decades, the hair-like papillae have been thought to be passive, static structures that simply increase the surface area of the tongue (5, 6).

In vivo studies on nectarivorous birds have shown that structures on the tongue tip are dynamic during feeding. In hummingbirds, the bifurcated tongue tips separate and the lamellae unfurl when the tongue is submerged in nectar (4). As the tongue is withdrawn, the lamellae roll inward and nectar is trapped within and between the tongue tips. The dynamic nectar trap in hummingbirds suggests that the hair-like papillae on nectar-feeding bat tongues may also be dynamic structures. To test this hypothesis, we investigated the anatomy and histology of the tongue tip in a nectar-feeding bat, *Glossophaga soricina*, and used high-speed video to visualize the movements of the tongue and papillae during nectar feeding.

Results

Tongue Morphology. The dorsal surface of the *G. soricina* tongue is covered with many lingual papillae (Fig. 1). Most of the

papillae are small filiform papillae, which consist of overlapping, serrated sheets of keratin. These small, pointed papillae give the middle region of the tongue a scale-like appearance (Fig. 1B). The dorsal and lateral surfaces of the tongue tip, however, are covered with elongated hair-like papillae, which are organized in discrete transverse rows along the distal third of the tongue. Each hair-like papilla is triangular in shape because it has a broad, flattened base and gradually tapers into a fine filamentous tip (Fig. 1C).

The *G. soricina* tongue is enveloped in fibrous connective tissue and stratified squamous epithelium, clearly seen in cross-section. At the tongue tip, the keratinized epithelium and fibrous connective tissue of the lateral tongue are elaborated into a set of finger-like projections (Fig. 2). These projections are the bases of the hair-like papillae, and they radiate from the main body of the tongue like spokes of a wheel. The core (i.e., medullary region) of the tongue is composed of orthogonally arranged muscle fibers (Fig. 2C). Horizontally and vertically oriented muscle fibers extend across the tongue's midline and longitudinally oriented fibers around the perimeter. This orthogonal arrangement of muscle fibers within the medullary region of the tongue is typical of mammals (7, 8).

Lingual arteries and veins are interspersed within these orthogonal arrays of skeletal muscle fibers. Together, the lingual arteries and veins form a vascular loop, which brings arterial blood to the tongue tip and returns venous blood to general circulation (Fig. 2B). In the proximal part of the tongue, paired deep lingual arteries run alongside the paired hypoglossal nerves (Fig. 2A). In the middle region of the tongue, the deep lingual arteries converge to form a single midline artery that continues into the tongue tip (Fig. 2B). Distally, this central artery is enlarged. Both the deep lingual and central arteries give rise to smaller blood vessels that extend into the dorsal and lateral regions of the tongue. All of the lingual arteries described above are completely surrounded by the tongue's horizontal and vertical muscle fibers (Fig. 2).

A specialized network of lingual veins drains blood from the tongue tip (Fig. 2). Near the base of the tongue, the deep lingual veins have large diameters and pass outside the muscular medullary region, close to the frenulum (Fig. 2A). In the middle region of the tongue, the deep lingual veins run on either side of the central artery and are embedded within the tongue's intrinsic muscle fibers. In this region, the endothelial lining of these veins is corrugated, suggesting that it could expand when filled with blood. There are no valves in the proximal and middle portions of the deep lingual veins.

The deep lingual veins are continuous with two vascular sinuses located in the tongue tip (Fig. 2). These enlarged sinuses extend longitudinally along the lateral margins of the tongue tip, just beneath the rows of hair-like papillae (Fig. 2B). The sinuses are thin-walled and irregularly shaped, suggesting that they are venous structures. The vascular sinuses communicate directly

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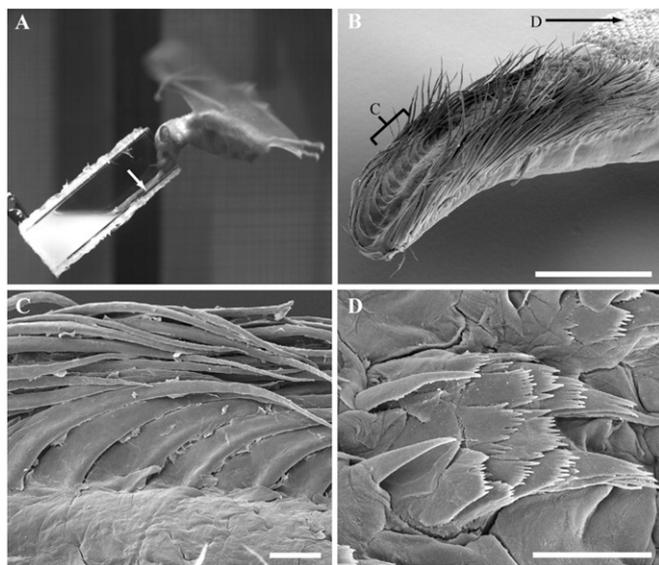


Fig. 1. Elongated tongue of a nectar-feeding bat, *G. soricina*, and its characteristic hair-like papillae. (A) *G. soricina* hovers in front of a feeder filled with artificial nectar and laps nectar with its long tongue. The white arrow highlights the distal tip of the tongue, which is covered in hair-like papillae. (B) Scanning electron micrograph of the tongue tip, showing a mop-like structure made of elongated lingual papillae. In this micrograph, the hair-like papillae are in their resting condition. (Scale bar: 1 mm.) (C) Scanning electron micrograph of the medial surface of the hair-like papillae, demonstrating that they are arranged in horizontal rows along the tongue tip. (Scale bar: 100 μ m.) (D) Scanning electron micrograph of small filiform papillae located on the middle and proximal regions of the dorsal surface of the tongue. (Scale bar: 30 μ m.)

with veins in the base of each hair-like papilla (Fig. 2C). These papillary veins are found only in the base of each papilla and do not extend into the filamentous tip. Red blood cells are visible within the lumina of the papillary veins, confirming that these spaces are vascular.

Observations from High-Speed Movies. A monochrome high-speed movie of nectar gathering in *G. soricina* shows that the hair-like papillae are not simple, static structures. Instead, the papillae become erect during nectar feeding, dynamically extending off the surface of the tongue with each lap (Movie S1). In the initial phases of tongue protrusion, the papillae are at rest; the bases of the papillae curve posteriorly and the filamentous tips lie flat against the surface of the tongue. As the tongue approaches maximum extension, the filamentous tips change their orientation to become perpendicular to the tongue's long axis (Movie S1). The hair-like papillae remain in their erected state throughout tongue retraction, and nectar is trapped between the transverse rows of papillae. Papilla erection always occurs just before maximum extension of the tongue and does not occur during initial tongue protrusion.

Our high-speed movies also show that papilla erection occurs in air when the tongue misses the nectar (Movie S2). When the tongue does not contact the nectar, the bases of the papillae still extend off the surface of the tongue. This observation shows that surface tension release does not drive the changes in tongue surface configuration in *G. soricina*, as it does in the hummingbird tongue (4). Therefore, a different mechanism must be responsible for papilla erection in bats. Based on the vascular morphology of the *G. soricina* tongue, we hypothesize that rapid blood flow into the vascular sinuses and papillary veins causes the papillae to become erect during nectar feeding.

A color high-speed movie shows increased blood flow to the vascular sinuses and engorgement of the papillary veins during nectar feeding (Fig. 3 and Movie S3). As the tongue first protrudes from the mouth and the papillae are at rest, the lateral

margins of the tongue tip overlying the vascular sinuses are pale pink, indicating that relatively little blood is contained within these vessels. As the tongue reaches maximum extension, the vascular sinuses and papillary veins engorge with blood and become bright red as the papillae become erect. Blood is temporarily trapped within these vessels and the papillae remain erect throughout tongue retraction (Fig. 3).

The papillae alternate between their rest and erect postures during each tongue cycle. At the beginning of tongue protrusion, the papillae rest flat against the tongue's surface, and as the

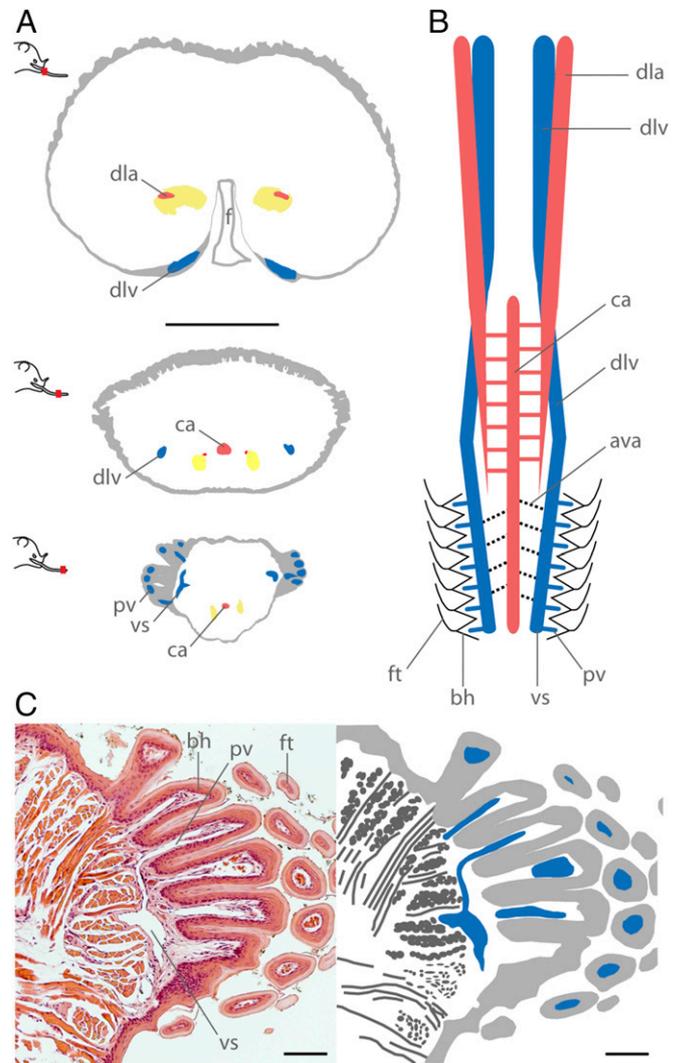


Fig. 2. Anatomy of the *G. soricina* tongue tip. (A) Line tracings of three transverse sections through the proximal (Upper), middle (Middle), and distal (Lower) region of the tongue. The dorsal surface of the tongue is directed up. These drawings highlight the location of the epidermis/dermis/papillae (light gray), skeletal muscle (white), hypoglossal nerve (yellow), arteries (red), and veins (blue). (Scale bar: 1 mm.) (B) Schematic of the arteries and veins within the *G. soricina* tongue. Arteries are shown in red and veins in blue. The dotted lines in the tongue tip illustrate the position of the arteriovenous anastomoses. (C) Transverse section and line tracing of the tongue tip showing the direct connection between the vascular sinus and papillary vein. This micrograph shows only the left side of the tongue tip. The color scheme in the line tracing matches the schematics shown above except, here, the orthogonally arranged skeletal muscle fibers are illustrated as dark gray lines. (Scale bar: 0.1 mm.) ava, arteriovenous anastomoses; bh, base of hair-like papilla; ca, central artery; dla, deep lingual artery; dlv, deep lingual vein; f, frenulum; ft, filamentous tip of hair-like papilla; pv, papillary vein; vs., vascular sinus.

collection, but the morphology and biomechanics of their tongue tips differ fundamentally. Together, these three systems could serve as valuable models for the development of miniature surgical robots that are flexible, can change length, and have dynamic surface configurations.

Materials and Methods

Tongue Morphology. To examine the external morphology of the lingual papillae, whole tongues were removed from three *G. soricina* and preserved in Karnovsky's fixative. Each tongue was dehydrated, critical point dried, and mounted on an aluminum stub. The tongues were coated in gold palladium and imaged with a Hitachi 2700 scanning electron microscope.

To examine the morphology of the intrinsic tongue muscles and blood vessels, whole tongues were excised from four *G. soricina* and preserved in neutral buffered formalin. Each tongue was separated into proximal, middle, and distal regions. Each region was then embedded in paraffin and sectioned along the transverse or longitudinal axis with a rotary microtome. All sections were stained with hematoxylin/eosin and imaged with a compound microscope (Nikon Eclipse e600 equipped with a Nikon DXM 12000C digital camera). Brightness and contrast of the digital images were adjusted with Adobe Photoshop.

High-Speed Movies of Nectar-Feeding Bats. Three *G. soricina* were filmed with a high-speed video camera (Photron Fastcam 1024 PCI or SA5; Vision Research Phantom v9 or v10). All tongue cycles were recorded at 500 frames per second, and fiber optic microscope lamps illuminated the tongue during lapping. Cardboard light shields were attached to the feeder to protect the bat's eyes from the light. The animals fed from a rectangular feeder filled with artificial nectar (17% mass/mass sucrose concentration). The rectangular shape of the feeder does not match the circular shape of a flower corolla, but this feeder design was necessary to prevent optical distortion.

Length and diameter of the tongue tip were measured *in vivo* in a total of 76 tongue cycles from three individual bats. Tongue tip length was measured as the distance between the horny papillae and the distal tip of the tongue. Tongue tip diameter was measured as the distance between the right and left sides of the tongue at the location of the horny papillae. Using ImageJ software (23), all distances were measured from movie frames at two specific points in the lapping cycle. Resting length and diameter were measured at the first emergence of the horny papillae from the mouth (Fig. 3; 10 ms). Extended tongue length and diameter were measured during tongue retraction, immediately after the tip exited the nectar interface (Fig. 3; 90 ms). Mean length and diameter were calculated for the three individuals at the two phases of lapping (i.e., start of protrusion; early in retraction), and these means were used to calculate an overall mean \pm SEM ($n = 3$ individuals) for each phase. A mixed-model ANOVA with individual and phase of the lapping cycle as factors was used to test for a significant change in tongue volume with elongation. Phase ($F = 17.8$; $df = 1$; $P < 0.0001$), individual ($F = 70.6$; $df = 2$; $P < 0.0001$), and phase by individual ($F = 3.6$; $df = 2$; $P = 0.0312$) were all significant at the $P < 0.05$ level.

Duration of events was measured by counting frames from high-speed movies. Mean durations were calculated for the three individual bats, and these means were used to calculate an overall mean \pm SEM ($n = 3$ individuals). The time to maximum tongue extension and the time required for blood to reach the tongue tip were measured in a total of 52 tongue cycles from three individuals. The duration of tongue protrusion was measured as the time from the first appearance of the tongue to the time just before the tongue reversed directions. The time required for blood to reach the papillary veins was measured from the first appearance of the tongue to the instant when the papillary veins became engorged with blood. The duration of a single tongue cycle (lap) was measured from the first appearance of the tongue tip to the moment when the tongue tip was fully retracted back into the mouth. The duration of a feeding bout (i.e., multiple tongue cycles in rapid succession) was measured in 29 bouts from three individuals. It was defined as the time from the first appearance of the tongue tip in the first lap to the time when the tongue retracted back into the mouth at the end of the last lap.

Postmortem Saline Injection Experiments. Neutral buffered saline was injected into the vascular sinuses of three excised tongues using a 31-gauge hypodermic needle and a 1-mL syringe. Once the papillae were erect, suture was tied around the circumference of the tongue. Photographs were taken before, during, and after saline injection with a digital camera mounted on a dissecting scope (Nikon Eclipse e600 with Nikon DXM 12000C digital camera). Adobe Photoshop was used to adjust the brightness and color balance of each photograph. Using ImageJ software, length and width of the artificially inflated tongues were measured using the same anatomical landmarks described in *High-Speed Movies of Nectar-Feeding Bats*.

All ligated tongues were preserved in Karnovsky's fixative and imaged with a scanning electron microscope. The scanning electron micrographs of the inflated tongues were manually stitched together in Adobe Photoshop to form composite images, and the brightness and contrast were adjusted in the composite image. Extra empty background was added to the left and center images in Fig. 4A. This modification did not affect the tongue tip lengths, but it was necessary to make the overall length of the left and center images match that in the right image.

Animal Welfare Statement. All activities involving live bats were approved by the Institutional Animal Care and Use Committee at Brown University (no. 1004016).

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