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Contribution of the maxillary muscles to proboscis movement in hawkmoths (Lepidoptera: Sphingidae)—an electrophysiological study

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Abstract

The role of the maxillary muscles in the uncoiling and coiling movements of hawkmoths (Sphingidae) has been examined by electromyogram recordings, combined with video analysis. The maxillary muscles of adult Lepidoptera can be divided into two groups, galeal and stipital muscles. The galea contains two basal muscles and two series of oblique longitudinal muscles, which run through the entire length of the galea. Three muscles insert on the stipes, taking their origin on the tentorium and on parts of the cranium and gena, respectively. Proboscis extension is initiated by an elevation of the galea base caused by the basal galeal muscles. The actual uncoiling of the proboscis spiral is accompanied by rapid compressions of the stipites which are caused by two of the stipital muscles. The study provides strong support for the hypothesis that uncoiling is brought about by an increase of hemolymph pressure by the stipites forcing hemolymph into the galeae. Recoiling is caused by the contraction of both sets of oblique longitudinal galeal muscles supported by elasticity of the galea cuticle. Finally, the remaining stipital muscle pulls down the galea base which brings the coiled proboscis back to its resting position where it is held in the U-shaped groove of the labium without further muscle activity.

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Keywords: Proboscis uncoiling and recoiling; Maxillary muscles; Electromyogram recordings; Sphingidae; Agrius; Acherontia; Manduca

1. Introduction

The spirally coiled and extendable proboscis, comprising the elongated maxillary galeae, is one of the most remarkable hallmarks of higher Lepidoptera (Lepidoptera-Glossata) and the mechanism of its movements is debated since Réaumur (1734). Numerous conflicting hypotheses on this subject have been proposed wherein the coiling and uncoiling of the proboscis has been variously attributed to intrinsic galeal musculature, hydrostatic pressure change within the galeal lumen, and elastic properties of the proboscis cuticle. The competing opinions can be classified as follows:

1. Both coiling and uncoiling is caused by two sets of

antagonistic intrinsic galeal muscles (Réaumur, 1734; Gerstfeld, 1853; Portier, 1949).

2. Coiling is caused by the muscles within the proboscis, whereas uncoiling is effected by elasticity of the proboscis cuticle (Burgess, 1880; Breitenbach, 1882; Lameere, 1938).

3. Coiling is caused and maintained by elasticity whereas muscle action uncoils the proboscis (Savigny, 1816; Kirbach, 1883; Hering, 1926; Weber, 1933; Pradhan and Aren, 1941; Forster, 1954; Vasudeva, 1956; Wigglesworth, 1964; Srivastava and Boga-wat, 1969; Eidmann, 1970).

4. Eastham and Eassa (1955) modified model 3 in that they supposed the intrinsic muscles to uncoil the proboscis rather indirectly by creating a transverse dorsal convexity which would produce cuticular tensions resulting in the uncoiling of the proboscis. Furthermore, they proposed that setting up an internal fluid pressure by closing up the proboscis hemocoel could be a prerequisite for the intrinsic muscles to become

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fully effective in producing the necessary cuticular tensions; a view shared by Schremmer (1961).

5. Hepburn (1971) conjectured that the same set of intrinsic galeal muscles could both coil and uncoil the proboscis, depending on the given hydrostatic pressure conditions within its lumen. Furthermore, he assumed that the mechanisms might differ between taxa.
6. The muscles inside the proboscis bring about coiling—possibly supported by elasticity—whereas uncoiling is a result of increased hydrostatic pressure caused by the cylindrically shaped maxillary stipites which are assumed to force hemolymph into the galeal lumen (Snodgrass, 1935; Schmitt, 1938; Imms, 1947; Bänziger, 1970, 1971, 1980; Krenn, 1990, 2000).

The variety of conflicting hypotheses is partly due to the complexity of the lepidopteran mouthparts, which differ between taxa and, thus, has led to considerable confusion and misunderstandings between investigators of different lepidopteran orders or families. The major difficulties, however, have arisen from methodical aspects. Most hypotheses are based on morphological and mechanical studies, some of which are partly inaccurate or incorrect, as already pointed out by Eastham and Eassa (1955). Experimental studies, however, are rare (e.g. Pradhan and Aren, 1941; Bänziger, 1971; Krenn, 1990, 2000), and thorough physiological investigations are lacking to date.

The aim of the present study is therefore to investigate the complete set of maxillary—proboscis intrinsic and extrinsic—muscles using electrophysiological methods. The role of the individual muscles in the coiling and uncoiling process was worked out by means of extracellular recording and stimulation techniques, combined with ablation experiments and video analysis. For optimum experimental accessibility, members of the hawkmoth family Sphingidae served as model organisms because of their particularly long or stout proboscides; amongst them the famous Malagasy *Xanthopan morgani praedicta* with a proboscis length of about 22 cm, and the death's head moth *Acherontia atropos* with its short and strong proboscis.

Experimentally validated knowledge of the muscular contribution to proboscis movement is an indispensable prerequisite to gain an improved understanding of proboscis mechanism and will allow a critical re-evaluation of the competing hypotheses.

2. Material and methods

2.1. Experimental animals

Eight hawkmoth species (Sphingidae) were studied: *Acherontia atropos* (L.), *Agrius cingulatus* (F.), *Agrius*

convolvuli (L.), *Manduca sexta* (L.), *Neococytius cluentius* (Cram.), *Panogena lingens* Butler, *Sphinx ligustri* L., and *Xanthopan morgani praedicta* Rothschild et Jordan. The animals came from Madagascar (*A. atropos*, *P. lingens*, *X. m. praedicta*), the Canary Islands (*A. atropos*), Costa Rica (*A. cingulatus*, *N. cluentius*), Teneriffa (*A. convolvuli*), and Southern Germany (*S. ligustri*). Eggs of *M. sexta* were obtained from an established laboratory stock from the Department of Animal Physiology, University of Marburg, Germany. The animals were kept in flight cages in a greenhouse under adequate climatic conditions and reared in the laboratory on their natural food plants with the exception of *M. sexta* being reared on an artificial diet (prescription after J. Schachtner, Marburg, personal communication).

2.2. Morphology

The morphology of the mouthparts and its musculature was studied by dissecting either freshly killed specimens submerged in Ringer solution (after Kaissling and Thorson, 1980) or specimens preserved in 70% ethanol. The preparations were performed with the aid of micro-dissecting tools under a Leica MZ8 stereomicroscope. To differentiate peripheral nerves from surrounding tissues, such as muscles or tracheae, the preparations were stained with Janus Green B (Yack, 1993).

2.3. Neuroanatomy

In order to delineate the innervation of the individual maxillary muscles for later stimulation and recording experiments, the head nervous system was cobalt stained using a slight modification of a technique described by Bacon and Strausfeld (1980).

The subesophageal (SEG) ganglion was filled using cobalt chloride filled glass cannulas. The animals were briefly anaesthetized with CO₂ and glued dorsum down on a plastic holder. The subesophageal ganglion was exposed from the ventral side by partly removing the labial plate. Care was taken to prevent injury of the air-sacs which restrict the head hemocoel to a narrow crevice system. This not only prevented desiccation but also diffusion of cobalt ions into the circulatory system. A fine glass cannula with a tip diameter of approximately 0.1 mm was filled with a 6–15% aqueous solution of cobalt chloride. The cannula was inserted into the subesophageal ganglion with the aid of a micromanipulator. The solution was applied passively by diffusion without application of pressure or current. Filling times were 24–36 h at 14–16 °C. After the withdrawal of the cannula, the animal was removed from the holder and decapitated after being anaesthetized once again. The cobalt ions were precipitated as cobalt sulfide by submerging the preparation in a 1% aqueous solution of ammonium sulfide for about 1 h. Finally, after removing

further cuticle, the preparation was dehydrated in an ethanol series and cleared in benzyl benzoate. The innervation was examined under a Leica MZ8 stereomicroscope and documented with a Leica MPS 60 camera.

2.4. Observations on the stipes movements

Since the stipites have been assumed to cause proboscis uncoiling by pumping hemolymph into the galeae by several investigators, their movements were studied in more detail. For this, heads of CO₂-anaesthetized animals were cut transversally at midpoint level of the stipites with a razor blade and transferred, cut surface upwards, to Ringer solution. As some preparations still showed spontaneous rhythmic activity of the stipital (and other) muscles following this treatment, it was possible to observe the changes in cross-sectional area of the stipites caused by muscular action.

2.5. Electrophysiology

2.5.1. Electromyogram recording

The maxillary muscles were examined by means of extracellular recordings during proboscis rest and movement, respectively. Preliminary tests had shown that proboscis uncoiling could be stimulated repeatedly in most specimens by applying either a 10–40% sucrose solution or 10–60% ethanol to the coiled proboscis with a pipette. A total of 113 specimens (47 *A. atropos*, 31 *M. sexta*, 13 *A. cingulatus*, 8 *S. ligustri*, 6 *P. lingens*, 3 *N. cluentius*, 3 *X. m. praedicta*, and 2 *A. convolvuli*) were recorded. The experiments were carried out in a Faraday cage within a climatic chamber at 22 °C (Fig. 1). The animals were anaesthetized and fixed on plastic holders as described

above. Insulated copper or silver wires with exposed tips and a tip diameter of 0.05 mm were used as recording electrodes. To make the recording sites accessible the labial palps were removed and the proboscis was held extended by a wire hook during the positioning of the electrodes. After perforating the cuticle with a steel needle both electrodes were inserted into the muscle of interest for bipolar recording and fixed on the cuticle with fixogum rubber cement (Marabu, Tamm, Germany). The electrodes were placed in the middle of the muscles; the galeal muscles were measured at different sites from the base to the distal region with the exception of the extreme tip. The signals were low- and highpass filtered and amplified using a custom-built differential preamplifier and digitized at sampling rates of 2–5 kHz using data acquisition hardware (MacAdios II, GW Instruments, Inc.) and software (Superscope II, GW Instruments, Inc.) for Macintosh. Alternatively, the signals were stored on DAT-recorder (Biologic DTR-1200) for later offline computer analysis. After the recording, the electrodes were cut off near the cuticle so that their tips remained within the muscle. Electrode position was checked by dissecting the animals under a stereomicroscope.

2.5.2. Video-recording

Parallel to the EMG recordings the proboscis movements were filmed and recorded using a Philips CCD camera and a video recorder. EMG and video recording were started simultaneously, both being synchronized via a video-recorded digital stop-watch, allowing later correlation of proboscis movement with muscle activity.

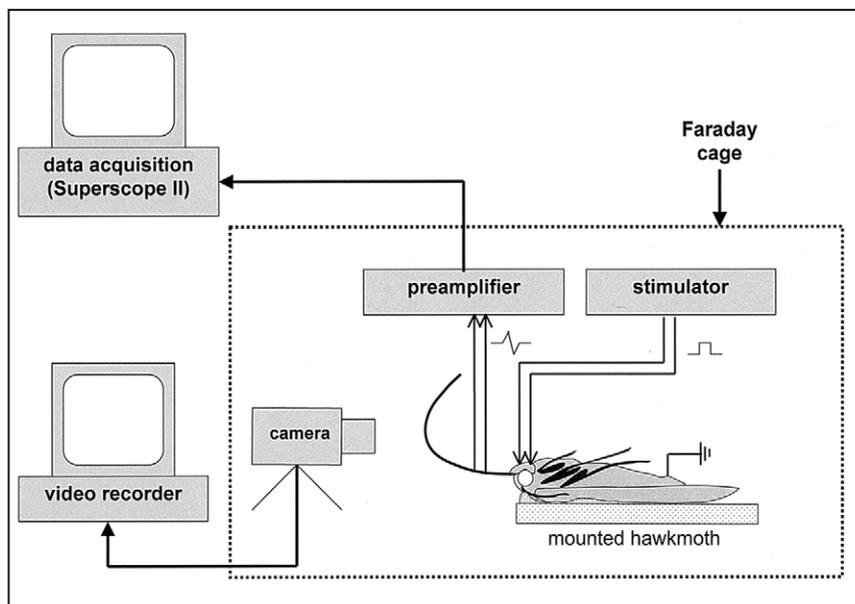


Fig. 1. Setup for extracellular myogram recording and nerve stimulation experiments.

2.5.3. Extracellular nerve stimulation

The maxillary nerve was stimulated electrically to evoke muscular responses. Electrolytically sharpened paired tungsten hooks were used as extracellular stimulating electrodes. The nerves were exposed by removing the membranous ventral side of the head and positioned on the hooks with the aid of a micromanipulator. A vaseline–oil mixture was placed around the hooked nerve to insulate it from hemolymph and surrounding tissues and to preserve it from desiccation. A custom-built stimulator was used to generate either single rectangular pulses of variable amplitude and duration or pulse trains of variable amplitude and frequency.

2.5.4. Ablation experiments

A number of ablation experiments were carried out and combined with EMG recording and nerve stimulation to test their effects on proboscis functionality. The experiments included the denervation of muscles and the perforation of the stipital or galeal lumen. To test the role of the intrinsic galeal muscles they were impaired by shock-freezing the proboscis in the uncoiled position using a cryospray (Kälte 75, Kontakt Chemie, CRC Industries Deutschland GmbH) containing dimethyl-ether. Again, the effects were tested by EMG recording and nerve stimulation 20 min and 1 day after thawing of the proboscis, respectively.

3. Results

3.1. Maxilla morphology

The proboscis of adult Lepidoptera is homologous to the maxillae, as has been shown by Savigny (1816), whereas the mandibles and the labium are highly reduced and do not contribute to food intake. Tillyard (1923) provided evidence that the proboscis is derived from the galeae. The general morphology of the lepidopteran mouthparts is described in common entomological textbooks and detailed morphological and anatomical studies are available for a number of species and families (e.g. Schmitt 1938; Pradhan and Aren 1941; Eastham and Eassa 1955; Eassa 1963a,b; Kristensen, 1968; Bänziger, 1970; Hepburn, 1971; Kristensen and Nielsen, 1981; Eaton, 1988; Krenn, 1990, 2000). Therefore, only a concise survey focussing on the maxillary muscles is given here. Since there is much discrepancy in the terminology of these muscles, due to the different views on their assumed function, a neutral nomenclature is used in this study (Table 1).

3.1.1. Maxillary muscles (Fig. 2)

The proboscis comprises the galeae, each of which contains two short basal muscles (GBM) and two series of intrinsic longitudinal oblique muscles—a ventral

(GVM) and a lateral (GLM) one (for detailed descriptions see Eastham and Eassa 1955, and Krenn, 2000). The stipes consist of a tubular and a flat part, with the latter bearing a broad apodeme. Two muscles insert on this apodeme, taking their origin broadly on the lateral surface of the anterior tentorial arm (STM) and on the gena (SGM), respectively. A third muscle arises posteriorly on the medial surface of the anterior tentorial arm (SPM) and inserts at the distal end of the stipes near the base of the galea. A longitudinal hinge lies within the lateral wall of the stipes tube. The lumen of the stipes tube is continuous with the galeal lumen and with the hemolymph space of the head cavity via a slit-like aperture.

3.1.2. The maxillary nerve

The maxillary nerves arise on either side of the anterior edge of the subesophageal ganglion, just beneath the smaller mandibular nerves (Fig. 3). Immediately after emerging from the SEG, the nerve gives off a branch which passes laterally beneath the anterior tentorial arm where it splits up to innervate the stipital muscles. The main branch, however, extends forwards, parallel to the anterior tentorial arm along the inner side of the SPM to enter the galea hemocoel where it runs beneath the galeal trachea to the proboscis tip. A detailed description of the head nervous system of *Manduca sexta* is given by Davis et al. (1996).

3.2. Stipes compression

Cross-sectional *in vivo* observations of the stipes movement indicate that two of the stipital muscles, STM and SGM, cause compression of the stipital tube (Fig. 4). These muscles, which seem to contract simultaneously, pull the flat stipes sclerite upwards in a dorsal and slightly lateral direction. This has two effects. First, the stipes apodeme is pressed against the outer side of the medial tube wall, thus acting as a flap valve and closing the slit-like aperture between the stipes and the head cavity. As a second result, the bottom of the tube is pulled upwards against the dorsal arch of the tube which remains fixed. This is enabled by the longitudinal fold within the lateral tube wall which seems to function as a hinge between the ventral and the dorsal part of the stipes tube. Consequently, the stipital lumen becomes constricted which can be observed as a decrease in cross-sectional area. When the muscles relax, the stipes goes back to its former shape and position, including the re-opening of the aperture. No muscular action is visible in this process. Re-extension seems to be due to elastic properties of the flexible hinge within the tube wall. The duration of one contraction–relaxation-cycle is about 0.5–2 s under these artificial conditions. Since the third stipital muscle, SPM, was cut in half between its origin and insertion points, these observations do not allow the

Table 1

Terminology of the maxillary muscles used by different investigators and their functions as worked out in the present study.

Muscle group	Muscle name	Function	Author
Stipital muscles	Posterior tentorial proboscis extensor		Schmitt, 1938
	Posterior tentorial muscle		Eastham and Eassa, 1955
	Stipes retractor		Eaton, 1988
	Posterior stipital muscle (SPM)	Retractor of galea base	This study
	Anterior tentorial proboscis extensor		Schmitt, 1938
	Anterior tentorial muscle		Eastham and Eassa, 1955
	Stipes extensor 1		Eaton, 1988
	Tentorial stipital muscle (STM)	Stipes compressor	This study
	Cranial proboscis extensor		Schmitt, 1938
	Cranial adductor muscle		Eastham and Eassa, 1955
Galeal muscles	Stipes extensor 2		Eaton, 1988
	Genal stipital muscle (SGM)	Stipes compressor	This study
	(Proximal and distal) Elevator muscles of the proboscis		Eastham and Eassa, 1955
	Galea extensor muscle		Eaton, 1988
	Basal galeal muscles (GBM)	Elevator of galea base	This study
	Primary oblique (retractor) muscles of the galea		Eastham and Eassa, 1955
	Galea retractor muscle		Eaton, 1988
	Lateral galeal muscles (GLM)	Proboscis coiling muscles	This study
	Secondary oblique (retractor) muscles of the galea		Eastham and Eassa, 1955
	Galea retractor muscle		Eaton, 1988
Ventral galeal muscles (GVM)	Proboscis coiling muscles	This study	

estimation of its possible contribution to stipes movement under natural conditions.

3.3. Proboscis movements and underlying muscle action

3.3.1. Proboscis extension

Proboscis extension consists of two separate movements, (1) the elevation of the galea base and (2) the uncoiling of the proboscis spiral (Fig. 5, top and middle trace). Galea base elevation is caused by the GBM and is usually maintained during the whole extension period. Uncoiling of the proboscis begins at the base and proceeds to the tip. It occurs in several distinct steps, each of which is accompanied by a compression of the stipital tubes. A compression is caused by a simultaneous contraction of the STM/SGM, as described above. The uncoiling velocity can vary and seems to be dependant on the frequency of the stipital compressions. At low frequencies of about 2–4 Hz, the stipites compress alternately (Fig. 5, inset). This is often observable at the final phase of uncoiling or during the maintenance of the extended position (Fig. 5, right of arrow in middle trace). During the actual rapid uncoiling process, however, the single STM/SGM bursts can even fuse, which causes the stipites to show an irregular ‘quivering’ pattern rather than distinct alternate compressions (Fig. 5, left of arrow in middle trace). It is difficult, however, to generalize this pattern, since the uncoiling seems to be a highly variable and adjustable process.

No SPM (Fig. 5, bottom trace) or GVM/GLM activity

(Fig. 6, middle and bottom trace) is measurable during extension.

In the intact proboscis, extension occurs in the sagittal plane. When the two galeae are separated, however, they uncoil with a slight turning to the sides, i.e. the left galea turns to the left and the right galea turns to the right. This is especially conspicuous at the tip region. Again, no GVM/GLM activity is detectable during these movements.

3.3.2. Proboscis recoiling

Proboscis recoiling usually (see below) begins with the stop of stipital activity and is caused by both galea intrinsic muscle sets, GLM and GVM (Fig. 6). GLM and GVM could be recorded separately only in *Acherontia atropos* because of the relative stoutness of its proboscis, but still some recordings contained crosstalk. In the other species, one bipolar electrode pair inside the galea picked up composite signals of both muscle groups due to their closeness. Recoiling begins at the tip of the proboscis and progresses to its base. It results in a tightly coiled spiral with consecutive coils being brought in close contact to each other. The initial phase of coiling often occurs without any detectable muscle activity and this always results in a loosely coiled spiral. Completion of coiling into a tight spiral is then effected again by the GVM/GLM. Simultaneous to this last phase of coiling or immediately following it, few alternating contractions of the SPM are measurable, which pull back the galea base and lower the coiled proboscis. As a result, the outer coil of the proboscis is brought in contact to the

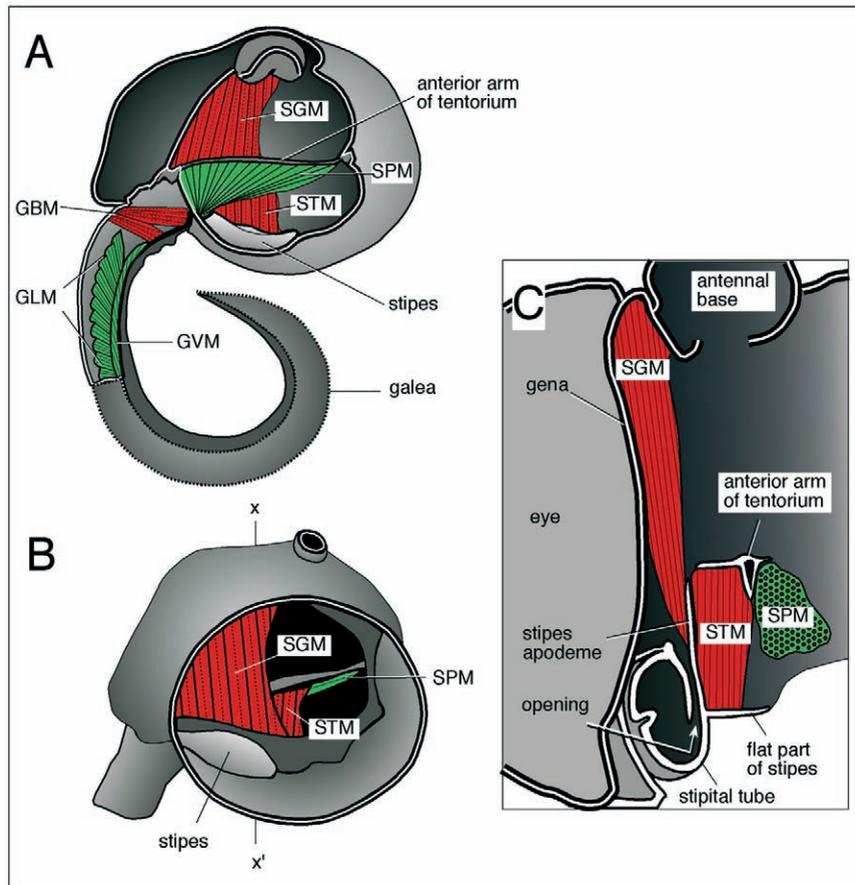


Fig. 2. Schematic views of the maxillary muscles of *Acherontia atropos*. Tracheal and nervous system and non-maxillary muscles removed. Muscles which are active during proboscis extension are depicted red, muscles which are involved in the coiling process are shown in green. (A) Mesal view of right head half, exposed by parasagittal cut. Galeal cuticle in the proximal region partly removed to show intrinsic muscles. (B) Lateral view of left head half, exposed by removal of eye. Galea cut basally. (C) Transverse section through stipital region of left head half, along the line X–X' in B. The arrow indicates the slit-like aperture of stipes into head hemocoel. GBM, basal galeal muscles; GLM, lateral oblique muscles of galea; GVM, ventral oblique muscles of galea; SGM, genal muscle of stipes; STM, anterior tentorial muscle of stipes; SPM, posterior tentorial muscle of stipes.

labial groove. Having reached this, the GVM/GLM relax and the proboscis spiral slightly re-opens to achieve its resting position. Under natural conditions, this position is maintained without any further muscle activity. In most experiments, however, contact of the proboscis with the labium was hindered by the stipital electrodes and in these cases, the animals repeatedly lowered the coiled proboscis without being able to maintain the resting position (Fig. 7).

3.3.3. Proboscis extension and concurrent recoiling

In situations of strain, especially after stimulation of extension by means of electric pulses or ethanol application to the proboscis, recoiling can commence before the stipes activity has ceased. In this case, STM/SGM and GLM/GVM activities are overlapping for a certain period of time (Fig. 8). Video analysis shows that during these overlapping activities the distal portion of the proboscis recoils while its proximal portion remains extended. This state can be maintained up to several

seconds during which the proboscis can become more uncoiled or more coiled, depending on the current activity dominance of either the stipital or the galeal muscle groups. If the GLM/GVM relax while STM/SGM activity is maintained, the proboscis again fully uncoils. If, in the reverse case, the stipital activity ceases while the galeal muscles remain active, the proboscis fully recoils. *Acherontia atropos* can even bend its short and stout proboscis downwards at an acute angle during extension at different lengths of the proboscis, i.e. the proboscis is fully extended proximal and distal to the bended point.

3.3.4. Stipital and galeal response to maxillary nerve stimulation

Electrical stimulation of the maxillary nerve can have different effects. When a single rectangular pulse (10 V, 1 ms) is applied while the proboscis is coiled loosely, it immediately coils tightly, in some cases accompanied or followed by one or a few stipes compressions. Appli-

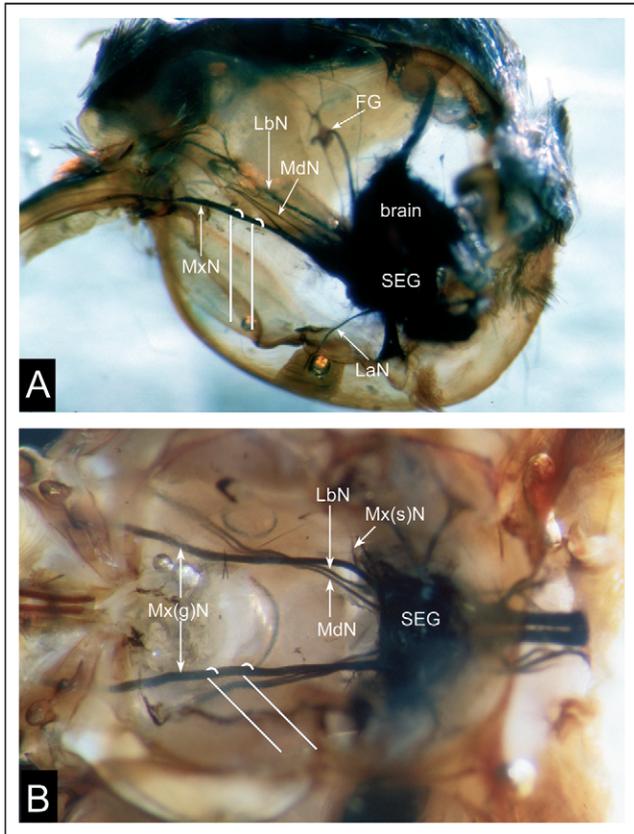


Fig. 3. Cobalt backfill of head nervous system of *Acherontia atropos*. (A) Longitudinal view by sagittal cut. (B) Ventral view by removal of ventral head membrane and labial plate. FG, frontal ganglion; LaN, nerve of labial palp; LbN, labral nerve; MdN, mandibular nerve; MxN, maxillary nerve; Mx(g)N, galeal branch of MxN; Mx(s)N, stipital branch of MxN; SEG, subesophageal ganglion. Position of bipolar stimulation electrodes on maxillary nerve is shown.

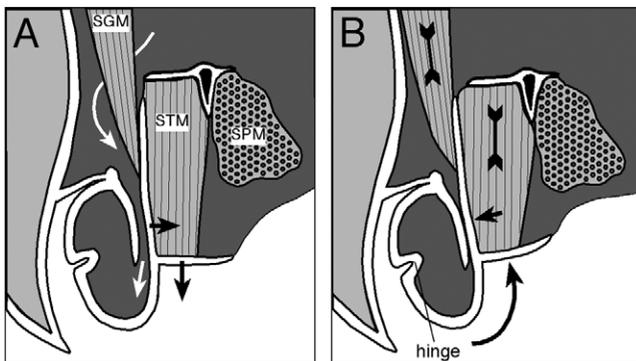


Fig. 4. Schematic transectional view of stipes compression. (A) SGM/STM relaxed. Stipital tube dilated and slit-like aperture between stipital lumen and head hemocoel opened (black arrows). Stipital tube is refilled with hemolymph from head hemocoel (white arrows) (B) SGM/STM contracted. Bottom of stipes is pulled up, enabled by flexible longitudinal hinge, and stipital apodeme is pressed against the inner tube wall (black arrows). Stipital tube compressed and aperture to head hemocoel closed. For details see text. Abbreviations as in Fig. 2C.

ation of a pulse train of about 500–2000 ms (10 V, 1 ms pulse duration, 9 ms pulse repetition rate) results in several rapid stipes compressions, accompanied by galea base elevation and proboscis extension, followed by tight recoiling and lowering of the galea base in most cases. Proboscis extension can exceed pulse train duration, but in most cases recoiling commences before stipital activity ceases, therefore showing the overlapping pattern described above.

3.3.5. Results of the ablation experiments

- (1) *Denervation of the galeal muscles.* If the maxillary nerve is stimulated with a pulse train after its galeal branch has been severed at its entrance to the galea, the stipes shows the same reactions as described, including the uncoiling of the proboscis, but the following recoiling into a tight spiral is always lacking.
- (2) *Removal of the stipital muscles.* If stipital compressions were excluded by cutting or completely removing the stipital muscles, maxillary nerve stimulation evoked one or several coiling movements.
- (3) *Perforation of the stipital tube.* Perforation of the stipital tube and following electrical stimulation of the maxillary nerve leads to stipes compressions while hemolymph can be observed to flow out of the incision. Stipital activity is accompanied by the elevation of the galea base but not by proboscis uncoiling. The ability of recoiling into a tight spiral is not affected.
- (4) *Abscission of the proboscis tip.* Cutting off the proboscis tip prior to nerve stimulation does not affect the stipital response, and the proboscis may still uncoil in some cases, but only briefly and incompletely, while hemolymph flows out of the wound. The proboscis is still able to recoil tightly. If a greater distal portion of the galea is removed, nerve stimulation does not lead to proboscis uncoiling anymore, although the stipital response is unchanged.
- (5) *Shock-freezing of the proboscis.* The proboscis was shock-frozen during extension with a cryo-spray. After thawing, the proboscis returned to the loosely coiled state. 20 min after thawing no GLM/GVM activity was detectable. One day after the experiment, the electrodes still registered no galeal muscle activity. Application of single pulses to the maxillary nerve evoked single stipital compressions but no coiling effects. Pulse trains lead to rapid stipital compressions and proboscis extension. After extension, the proboscis slowly turned back into a loosely coiled spiral but never recoiled tightly.

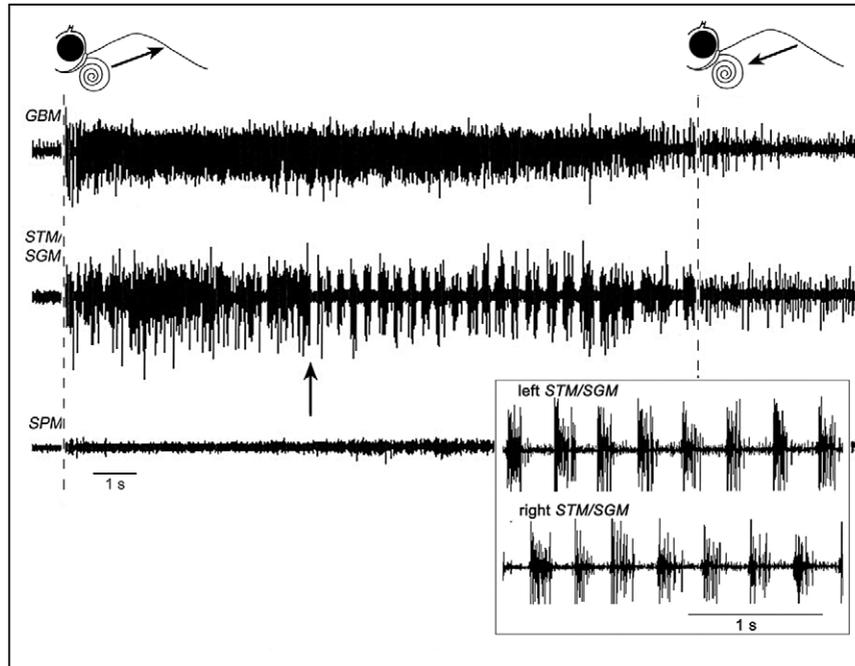


Fig. 5. Extension sequence (between dashed lines) in *Manduca sexta*. Electromyograms of the GBM, STM/SGM and SPM of the right head half. Extension consists of two separate movements, (1) elevation of the galea base by contraction of the GBM (top trace), and (2) uncoiling of the proboscis, accompanied by stipital compressions caused by the STM/SGM (middle trace). During uncoiling the stipital compressions fuse, when the proboscis is fully extended (arrow) the stipites compress alternately with a frequency of about 3 Hz (inset). The SPM is not involved in the extension process (bottom trace). Abbreviations as in Fig. 2.

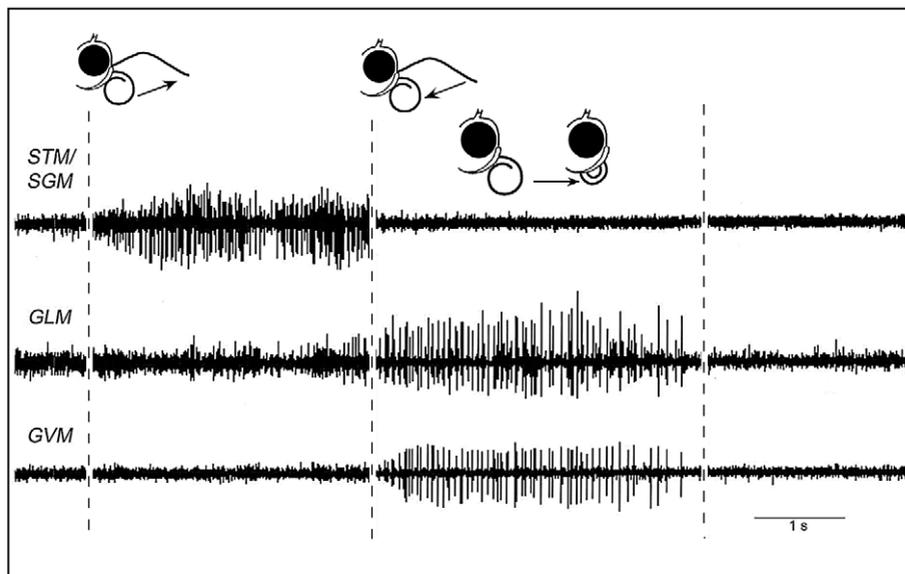


Fig. 6. Uncoiling (between left and middle dashed line) and subsequent recoiling (between middle and right dashed line) in *Acherontia atropos*. EMGs of STM/SGM (top trace), GLM (middle trace), and GVM (bottom trace) of left head half. GLM and GVM electrodes were placed in the distal third of the galea. Recoiling begins with the cease of stipital activity and is caused by both series of intrinsic oblique galeal muscles. Abbreviations as in Fig. 2.

4. Discussion

The maxillary muscles, especially the intrinsic galeal ones, have been made responsible for coiling and uncoiling of the lepidopteran proboscis in a variety of

competing hypotheses and this disagreement on their function has led to a confusing terminology (Table 1). It should be possible now, in the light of the new experimental findings, to designate them more adequately with regard to their actual functions. There has been much

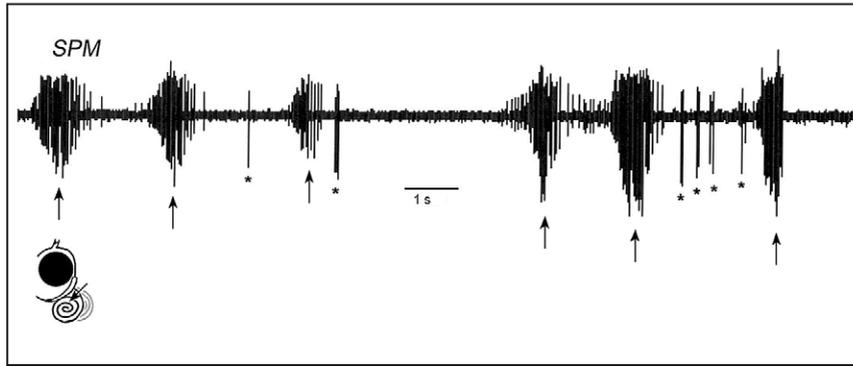


Fig. 7. Repeated lowering of the coiled proboscis in *Manduca sexta*, caused by contractions of the SPM (arrows). EMG of SPM of right head half. Maintenance of the resting position was mechanically hindered by the recording electrodes. Further explanation see text. Abbreviations as in Fig. 2. Asterisks mark crosstalk from pharynx musculature.

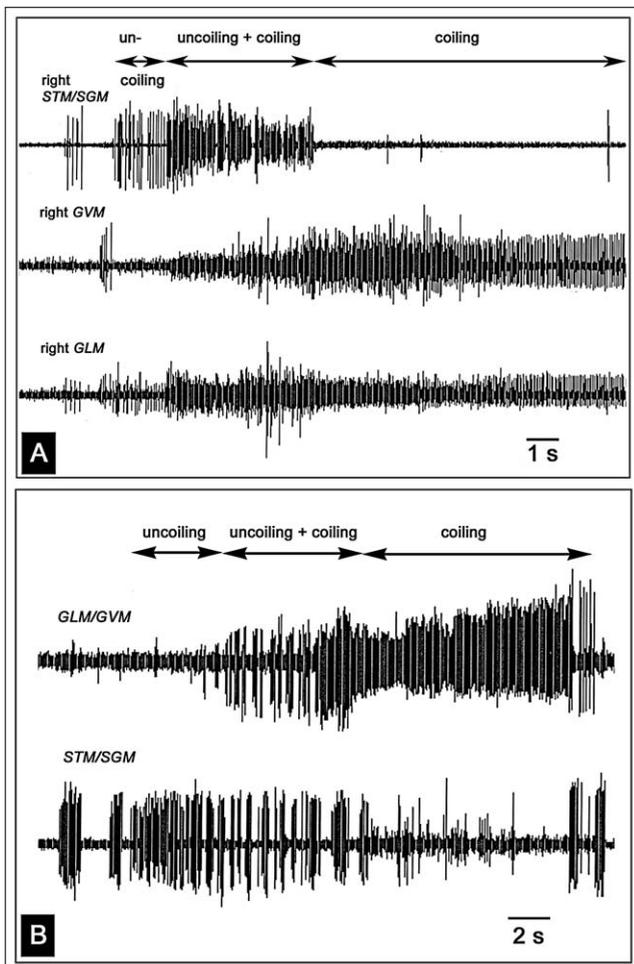


Fig. 8. Two examples of overlapping STM/SGM and GLM/GVM activity, resulting in simultaneous uncoiling and coiling in *Acherontia atropos*. GLM/GVM electrodes were placed in the middle of the galea in A and 5 mm proximally to the galea tip in B. Exclusive activity of STM/SGM causes stipes compressions, accompanied by proboscis uncoiling, sole GLM/GVM activity causes proboscis coiling. Simultaneous activity of both systems results in concurrent uncoiling and coiling movements. For further explanation see text. Abbreviations as in Fig. 2.

consensus that the basal galeal muscles, GBM, serve as *elevators of the galea base*. However, the two series of longitudinal oblique muscles, GLM and GVM, have produced a controversy. Electromyography has clearly shown that they are both involved in the coiling process, and so they can be named as *lateral* and *ventral coiling muscles of the proboscis*, respectively. Two of the three pairs of extrinsic or stipital muscles, named anterior tentorial and genal stipital muscles in the present study (STM and SGM) have been shown to cause the stipital compressions which occur during proboscis extension, and so their correct designation from a functional point of view would be *tentorial* and *genal compressor muscles of the stipes*. The remaining extrinsic maxillary muscle, SPM or posterior tentorial stipital muscle, pulls the galea base backwards and downwards in the final phase of coiling and therefore can be designated as *retractor of the galea base*.

The electromyogram recordings as well as the ablation experiments carried out in the present study provide strong support for the hemolymph pressure theory introduced by Snodgrass (1935) and refined by Schmitt (1938), Bänziger (1971) and Krenn (1990, 2000). All other models explain incorrectly the mechanism of uncoiling, some of them, however, provide correct contributions to the coiling mechanism. For this reason, they will be discussed in more detail in the following sections.

4.1. Proboscis extension

Extension starts with the elevation of the galea base. That this process is caused by the muscles inside the galea base seems plausible and almost trivial and thus has never been doubted explicitly by any author (although ignored by some). Interestingly, this movement is not restricted to proboscis extension but is also involved in a reflexory answer to acoustic stimulation in some acherontiine species, as has been shown recently

by electrophysiological measurements (Göpfert and Wasserthal, 1999).

It is the mechanism of the actual uncoiling process that has provoked controversy. Snodgrass (1935) first assumed “that the proboscis is extended by blood pressure, in the same way that a paper ‘snake’ is unrolled by inflating it”, and he continued “[T]he mechanism for creating the assumed blood pressure, however, is not evident”. It was Schmitt (1938) then who regarded the stipites to create the blood pressure needed for extension by acting as pumping organs which force hemolymph into the galeae; a conjecture which could be supported by observations and experiments of Bänziger (1971) and by vital staining experiments of Krenn (1990). Schmitt proposed the three pairs of stipital muscles would cause the necessary stipital compressions, an assumption which is only partly confirmed by the present study. Two of the stipital muscles, STM and SGM (*anterior tentorial proboscis extensor* and *cranial proboscis extensor* according to Schmitt) indeed cause stipital compressions in the way described in detail in this study. The third muscle, SPM (*posterior tentorial proboscis extensor* of Schmitt), however, is not involved in the extension but in the final phase of the recoiling process. It is notable, in this respect that the fruit piercing noctuid *Calpe (Calyptra) thalictri* and its blood sucking relative *Calyptra eustriga* have four instead of three stipital muscle pairs, two of which are responsible for the stipital compressions as in the other Lepidoptera, but the remaining two function as galea retractor and protractor, respectively (Bänziger, 1970, 1980). These latter serve in the piercing process by causing antiparallel movements of the galeae. Since the proboscides of these species possess barbs which are, according to Bänziger, erected by increased hemolymph pressure during extension, they provide indirect support for the blood pressure theory; although direct pressure measurements within the galeal hemocoel are lacking to date.

Krenn (1990) studied proboscis movement by means of cinematography and noticed that proboscis extension occurs simultaneously in all regions. This is in contrast to descriptions of Snodgrass (1935) and Eastham and Eassa (1955), according to whose uncoiling runs successively from the base to the tip, a pattern which is in congruence with video analyses of the present study. Furthermore, Krenn (1990), in concordance with Bänziger (1971), observed that extension occurs in several distinct steps, each of which is accompanied by a simultaneous compression of both stipites. This pattern turned out to be more complex, however, since the extension process is not stereotypical, and the extension velocity, for instance, which is presumably a consequence of stipital compression frequency, seems to be highly flexible and adjustable.

No intrinsic longitudinal galeal muscle activity was detectable during uncoiling. This is also true for the

delicate sideway moving of the proboscis tip, which is often observable during foraging. This refutes the assumption of Bänziger (1970) that the GVM (“secondary oblique muscles” according to the author) could be responsible for the lateral movements of the proboscis. Experimentally separated galeae slightly move sideways during uncoiling, and as no muscle activity is involved, this can be expected to be due to asymmetrical properties of the galea cuticle. It can be assumed, therefore, that the sideway movements of the tip in the intact proboscis are caused by asymmetrical pressure conditions inside the galeae. For instance, more powerful and/or more rapid compressions of the left stipes, compared to the right one, would cause the whole proboscis tip to bend to the left and vice versa; a mechanism already considered by Krenn (1990).

The competing hypothesis that proboscis extension is brought about by intrinsic galeal muscle action is not supported by the present study. Réaumur (1734) regarded both coiling and uncoiling as being caused by two antagonistic sets of muscles inside the proboscis, but according to Eastham and Eassa (1955), he “confused the cuticular annulations [of the proboscis wall] with ring muscles to which he attributed coiling, [and] he may perhaps be regarded as among those who claimed the intrinsic longitudinal muscles to be the cause of extension”. Gerstfeld (1853) and Portier (1949) seem to have uncritically adopted Réaumur’s hypothesis. Pradhan and Aren (1941) assumed that the intrinsic galeal muscles would uncoil the proboscis and elasticity would be responsible for coiling. They based their model on two assumptions. First, they claimed that the stipital muscles, which Schmitt (1938) described “as being inserted on a ‘median flat sclerite’, are actually inserted *within* the cavity of the stipes [...and thus] [I]t is difficult to see how the opening of the stipital cavity can become liquid-tight, being closed by the contraction of the muscles coming out of the same opening into the head-cavity”. This, however, is in contrast to the findings of all other authors and since they enclosed no illustrations to support their assertion, it is difficult to comprehend. Second, the authors postulated, “that the elasticity of the proboscis is lodged within the posterior [ventral] wall, which functions as the spring-wire of the toy ‘paper snake’ [according to Snodgrass], and keeps the proboscis coiled, whereas the anterior [dorsal] wall is foldable and admits of uncoiling”. Again, this contrasts to later findings. Eastham and Eassa (1955) provided evidence that the elastic properties of the proboscis are located within ‘an exocuticular dorsal longitudinal bar’ of each galea, a finding which was confirmed experimentally by Bänziger (1971). Hepburn (1971) found that this dorsal bar consists of resilin, a highly elastic rubber-like protein discovered in insect cuticle by Weis-Fogh (1960), which occurs in cuticle areas of great flexibility such as tendons, leg-joints and wing-hinges. Pradhan and Aren

(1941) constructed an artificial clock-spring model which they thought would prove their hypothesis, but since it is based on a false premise its validity must be doubted. The most elaborated of the ‘muscular extension hypotheses’ is that of Eastham and Eassa (1955), based on a study of *Pieris brassicae*. These authors explained proboscis extension as a “consequence of [a] dorsal convexity” of the galea wall which is caused by the contraction of the intrinsic galeal muscles under the precondition of a “haemocoelic turgidity maintained by a stipital valve”. This model has been critically reviewed by Bänziger (1971) and Krenn (1990, 2000). The first author demonstrated that some experiments carried out by Eastham and Eassa were either inadequate to test their hypothesis or were interpreted incorrectly. Krenn (1990) pointed out that Eastham and Eassa incorrectly compared cross-sections of different proboscis regions to prove the postulated change in shape during extension.

4.2. Proboscis coiling

Coiling of the proboscis has been at least partly attributed to elasticity of the galea cuticle by most researchers. Only Burgess (1880), Breitenbach (1882) and Lameere (1938) assumed that elasticity would uncoil the proboscis. However, this is not supported experimentally in any way. According to a great number of authors (Savigny, 1816; Kirbach, 1883; Hering, 1926; Weber, 1933; Pradhan and Aren, 1941; Forster, 1954; Eastham and Eassa, 1955; Vasudeva, 1956; Wigglesworth, 1964; Srivastava and Bogawat, 1969; Eidmann, 1970) elasticity is the only agent of coiling. It was Bänziger (1971) then, who produced experimental evidence that elasticity alone could only loosely coil the proboscis, whereas a functioning intrinsic musculature is required for tight coiling; a finding which is confirmed by shock-freezing experiments of Krenn (2000) as well as by electromyography in the present paper. The EMG recordings show that coiling is caused by *both* series of oblique longitudinal galeal muscles, GLM and GVM, thus excluding the possibility that they might act as antagonists. Hepburn (1971) assumed “the possibilities of the galeal muscles being used for both extension and recoil given varying conditions of hydrostatic pressure”. Again, this is contradicted by the EMG recordings, which show that the galeal muscles do not only coil the proboscis during the absence of stipital activity, but also during the simultaneous activity of stipital muscles, which results in the concurrent coiling and uncoiling movements described in detail in this paper. It is not clear whether this behavior is merely artificial or of biological relevance, at least in *Acherontia atropos* it can be assumed to be used in the opening of honeycombs.

Krenn (1990) described alternating compressions of the stipites in the last stage of the coiling process which do not lead to uncoiling. Electromyography shows that

these movements are due to the activity of the third pair of stipital muscles, SPM, which is not included in the extension process but helps the proboscis to attain its resting position by lowering the galea base. It cannot be excluded that these muscles do not only serve in the interlocking process of the coiled proboscis but also “could open the stipital valve to allow back-flow of the hemolymph into the head”, as assumed by Krenn (1990).

The hemolymph pressure theory as well as the coiling function of the intrinsic galeal musculature are not only supported experimentally, but also by phylogenetic considerations. The proboscides in the basal glossatan taxa, including *Eriocrania*, do not possess internal muscles (Kristensen, 1968), and, according to this author, “the blood-pressure theory apparently gives the only possible explanation of the extension”. It can be assumed that elasticity is sufficient to coil the simple proboscides of these groups. Basal (‘non-ditrysiian’) Myoglossata have simple intrinsic galeal muscles consisting of longitudinal fibers close to the ventral wall—in contrast to the numerous oblique muscles of Ditrysia—which are assumed by Kristensen and Nielsen (1981) to “function in conjunction with elastic forces as the antagonists to hydrostatic forces”. If so, these muscles would have simply retained their original function in higher myoglossatan Lepidoptera, despite their morphological diversification that occurred in these groups.

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