The pars media/interna anatomy and histology of the cricothyroid muscle

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Abstract

For proper understanding of function a thorough understanding of structure is necessary. This applies to the larynx. The pars interna/media (PIM) is a small muscle found in the human larynx that is not described in contemporary literature on laryngeal structure. Our objective was to describe the PIM's anatomy. Thirteen human and Rhesus macaque larynges obtained from post-mortem examination were cleaned and preserved. Exposure of the PIM was through a lateral disarticulation of the cricothyroid joint and reflection of the cricothyroid muscle. In the human, the PIM was found to be strap-like in form and to have two bellies with attachments to the medial surface of the thyroid cartilage at the root of the inferior horn and anterio-superior cricoid arch. It appears to be innervated by a middle division, vestibular branch, of the internal superior laryngeal nerve. The average fiber diameter is 40 microns. Its Type I to Type II fiber ratio places it within the range of other intrinsic laryngeal muscles. A muscle spindle was identified in medial bundle at the PIM's thyroid attachment. Thyroid medial surface attachment is within few millimeters of the muscular process of the arytenoid cartilage. In the Rhesus, the PIM appears to be a twisted cone, with a huge medial thyroid lamina surface attachment, relative to the human. These data show that the PIM is a robust muscle that deserves attention anatomically. Its orientation within the thyroid and non-recurrent laryngeal nerve innervations of the human PIM may place it in the vocal fold tensor group rather than the laryngeal sphincter group. The PIM found in the Rhesus larynx may have a power role in laryngeal skeletal stability. Electromyographic examination of the PIM in the Rhesus larynx may help elucidate its physiology and help to elaborate human physiology.

Keywords: cricothyroid muscle, laryngeal anatomy, laryngeal function, muscle anatomy, muscle histology

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1. Introduction

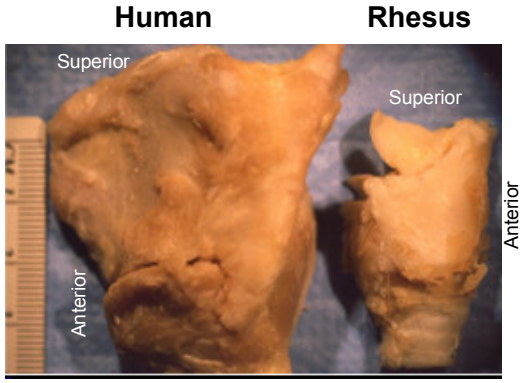
The pars interna/media (PIM) of the cricothyroid (CT) has been infrequently mentioned in published literature and one of these mentions was to debate its existence 1,2,3. Von Mayet and Mundnich (1) reported that the PIM was present in their dissections but did not report on the muscle's attachments, relationships, innervations, histology or histochemistry. Kucinski et al (2) dissected several human larynges and considered the PIM little more than anatomical variation of the cricothyroid proper. Tschiassney (4) examined extensively the cricothyroid muscle but did not mention the PIM. Work performed by this author and reported in the early 1990s confirmed the existence of the PIM in human larynges. Recently, Dr. Ira Sanders presented data from dissections in the dog showing a similar muscle based on laryngeal attachments (5). Additionally, there are no reports of a PIM-like muscle in the primate larynx (6,7). We initiated a new set of investigations to identify the physical nature of the PIM in both the human and the primate.

2. Materials and Methods

2:1: Human

2:1:1 Specimen harvest
Thirteen human larynges (twenty-six hemi-larynges), free of pathology, were obtained from autopsy. Both sexes, four female and nine males, represented ages ranged from fifty-eight to ninety-one. The harvest of human larynges was made possible by the approval of a protocol for anonymous donor tissue by the Boston University School of Medicine's Institutional Review Board for Human Research (X4207; 105-106).

Figure 1.



Lateral views

Specimen processing
After removal of the larynx from the cadaver, extraneous tissues were removed from the specimen and then mid-line bisected. Specimens were fixed in a 10% buffered formalin fixative for at least 24 hours. After fixation, each specimen was rinsed under running tap water. Each specimen was dissected according to protocols designed to preserve neural, vascular, and muscular anatomy. Specimens were placed on a Styrofoam stage under the dissecting microscope with 6x to 30x magnification range. Specimens were moistened, when necessary, with a solution of normal saline and glycerin. PIM

access was obtained by a lateral approach which consisted of: (1) reflecting the cricothyroid muscle by cutting away the muscle with an iris knife from its thyroid cartilage, inferior border attachment, including the anterior surface of the inferior horn; (2) and disarticulation of the cricothyroid joint by carefully cutting the joint capsule's four ligaments, including the medial ligament. The entire thyroid lamina can then be elevated carefully to reveal the muscles associated with the glottis, including the PIM. In this way, preservation of the PIM, as well as other laryngeal structures, was easily accomplished. (Figure 1) Each stage of dissection was documented with notes, line and schematic drawings and photography. In all specimens, after noting attachments, muscle orientation, and position relative to other muscles within the larynx, PIM were measured for length, width and thickness and examined for the number of bellies per muscle.

In one specimen the PIM was removed from its attachments, sectioned longitudinally, and processed for histological examination that included routine hematoxylin and eosin and Mallory's trichome for connective tissue. Also, two histochemical stains, silver stain for nerve fibers and myoneural junctions and succinate dehydrogenase to differentiate muscle fiber types based on mitochondrial concentration, were used. Slides were examined visually for general morphology, muscle fiber types, morphometric estimate of muscle fiber diameter, and the presence of muscle spindles. An attempt was made to estimate muscle spindle length by noting the number of slides showing spindle cross section, then adding the thickness of each section to obtain an approximate length (number of slides x 15 microns). Morphometric estimates of muscle fiber and spindle diameters were made by a linear diameter estimate on an Optima 5.2 computerized morphometric system at the Mallory Institute, Boston Medical Center, in Boston, MA. To estimate number of fibers types, four random fields of a single stained section (30x magnification) were examined by counting the number of light and dark muscle fibers. Both absolute numbers and a ratio of light to dark fibers types were estimated with this method. The above data were compared with other reports on histological and histochemical descriptions of other intrinsic laryngeal muscles.

2:2:1 RESULTS

2:2:1:1 Gross structure

The PIM has a ribbon-like anterior attachment arising from the superolateral surface of the anterior cricoid arch, underneath and medial to the fibers of the pars recta of the cricothyroid muscle. The PIM's course causes it to be overlaid, but entirely separate from, the deep anterior fibers of the CT's pars recta. The PIM also overlays the lateral cricoarytenoid's more anterior attachment on the superior surface of the lateral cricoid arch. The inferior border of the thyroid lamina is flattened in a roughly triangular shape and fits closely with the flattened surface of the cricoid arch to form a thin intercartilagenous compartment through which the PIM courses. Emerging from the intercartilagenous compartment, the two bellies of the PIM arise from a common tendon course posteriorly by unwinding approximately 90 degrees, one-quarter turn, laterally to emerge from underneath the inferior border of the thyroid lamina. The two bellies of the PIM continue posteriorly, in parallel, remaining close the medial surface of the thyroid lamina, to attach on the medial surface of the, and within few mm, of the anterior of the root of the inferior horn. Both bundles merge in a fusiform shape, one belly medial into the other. The PIM's posterior attachment is 2-3 mm lateral to the muscular process of the arytenoid cartilage. (Figure 2 and 3).

Innervations of the PIM appear to be supplied by the middle division of the internal branch of the superior laryngeal nerve. The nomenclature for the divisions was derived from Sanders (5). According to Sanders, the internal superior laryngeal nerve divides into a superior, or epiglottic, division, a middle, or vestibular, division and an inferior, arytenoid-posterior cricoid anastomotic, division. Ramification of the internal superior laryngeal nerve occurs upon entering the space between the laryngeal viscera and the laryngeal skeleton in a fatty bursa. The middle division consistently gives off a branch that crosses the space between the tissue of the sphincteric muscles and thyroid lamina where it penetrates a foramen on its medial surface. At the foramen, a small branch is given off that descends inferiorly in a neurovascular bundle. Nerve fibers and blood vessels penetrate the bellies of the PIM at the midpoint of the muscle's length, a presumed neurovascular hilus. (Figure 3). The average dimensions of the PIM are: length = 258 mm (S.D. 23 mm), width = 4.55 mm (S.D. 0.50 mm), thickness, each bundle, = 3.33 mm (S.D. 0.57). All PIMs except one had two bellies. (Table I).

Figure 2.

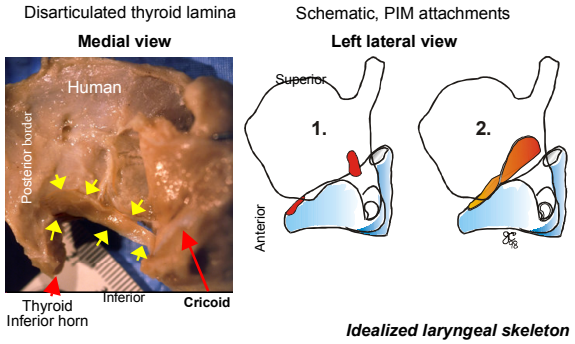
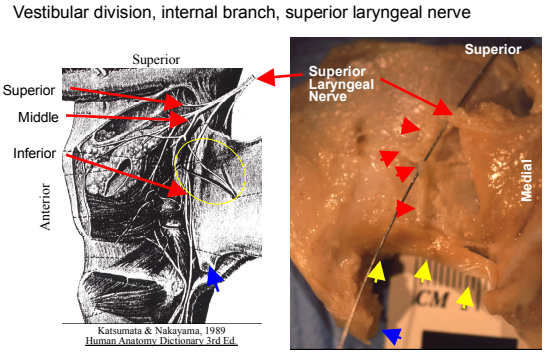


Figure 3.



2:2:1:2 Histology

PIM histology revealed muscle fibers endomysially grouped together as fascicles and bundles of fascicles perimysially grouped together with connective tissue septa containing blood vessels and nerves. (Figure 5). A single nerve fiber was identified as it entered the muscle, coursing from the medial surface of the middle of the belly before dividing in to several

motor branches. A single muscle spindle was identified in the posterior end of the medial bundle. Its length was estimated to be approximately 1.8 mm, with a maximum diameter of approximately 0.8 mm. The spindle contained four intrafusal fibers surrounded by an easily identified capsule. (Figure 5, inset).

2:2:1:3 Histochemistry

Sliver-stained sections were examined for nerve fibers and myoneural junctions. Three nerve fibers could be seen to run in the medial bundle, dividing it into three sections. These fibers do not appear to travel in axonal bundles surrounded by a supportive sheath. Rather, the fibers occur singly with their junctions making close contact with muscle cells. However, no multiterminal innervation patterns were identified. Closer examination revealed myoneural junctions but also blebs on the fibers, indicating post-mortem degradation. (Figure 4).

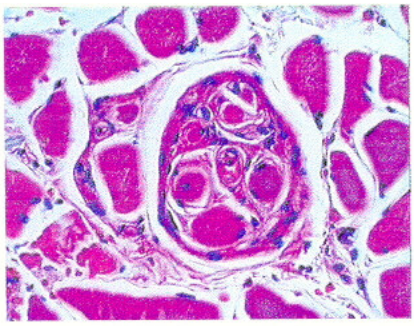
PIM fiber types from succinate dehydrogenase staining revealed PIM histochemistry similar to other intrinsic laryngeal muscles. PIM ratio of Type I to Type II (TI/TII) was found to be 0.801. By comparison, limb skeletal muscle ratio is reported to be 0.632. Other intrinsic laryngeal muscles' TI/TII ratio is 0.981 for the posterior cricoarytenoid, 0.987 for the cricothyroid, and 0.889 for the lateral cricoarytenoid as previously described (7). Average PIM fiber diameter was 45 microns, whereas posterior cricoarytenoid was 40 microns, cricothyroid was 40 microns, and lateral cricoarytenoid was 50 microns. The greatest number of fibers was at the average size for each. This is consistent with previous work by others (8). See Table I.

Table 1.

	Posterior cricoarytenoid		Cricothyroid (recta & oblique)		Lateral cricoarytenoid		Pars Interna/Media	
Size	Type 1	Type 2	Type 1	Type 2	Type 1	Type 2	Type 1	Type
0	0	0	0	0	0	0	0	0
5	1	1	0	0	10	8	0	0
10	4	2	1	2	10	9	3	1
15	5	3	0	3	12	12	8	5
20	8	7	5	5	18	17	10	7
25	12	12	12	12	20	19	18	12
30	17	15	17	15	24	23	22	20
35	22	23	22	23	26	23	24	20
40	26	22	30	27	30	27	27	24
45	17	22	17	22	37	30	30	24
50	15	16	20	20	40	38	22	20
55	10	8	15	15	33	30	12	9
60	9	8	7	6	22	21	8	7
65	5	6	6	5	19	17	4	3
70	3	2	3	3	12	10	2	1
75	1	1	4	4	9	7	1	0
80	0	0	2	1	4	4	0	0
85	0	0	1	1	4	2	0	0
90	0	0	0	0	2	1	0	0
95	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0
	265	298	162	164	265	298	191	153

	Posterior cricoarytenoid	Cricothyroid (recta & oblique)	Lateral cricoarytenoid	Pars Interna/Media
Ratio	*0.98*	*0.99*	*0.89*	*0.8*

Figure 4.

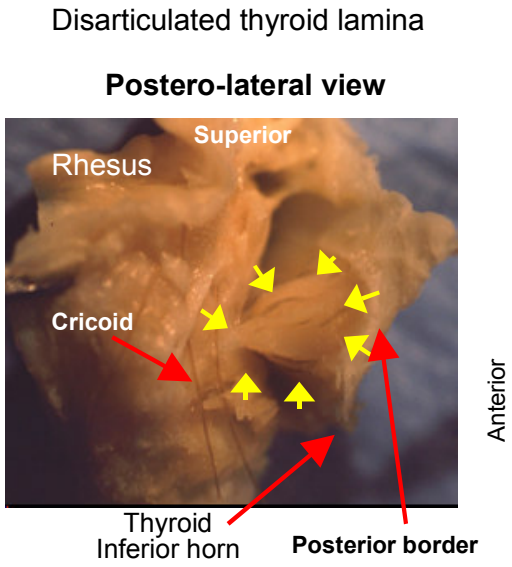


3:1: Rhesus Macaque

3:1:1 Specimen harvest

Thirteen rhesus larynges (twenty-six hemi-larynges), free of pathology, were obtained from autopsy. Sexes, six female and seven males, with an age range of five to thirty-six months. The harvest of rhesus larynges was made possible by the approval of the primary investigator, Dr. Douglas Rosene, whose brain aging study did not in any way affect the structure or function of the organ of interest.

Figure 5.



3:1:2 Specimen processing

Processing of rhesus larynges was identical as that report above for the human. As of this date, no descriptions of the PIM in the Rhesus exist in the primate anatomical literature.

3:2 RESULTS

3:2:1 Gross structure

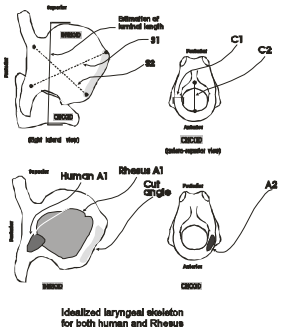
The PIM has a cone-like anterior attachment arising from the superolateral surface of the anterior cricoid arch, medial to the fibers of the pars recta of the cricothyroid muscle. Unlike the

human PIM, where its course causes it to be overlaid, but entirely separate from, the deep anterior fibers of the CT's pars recta, the Rhesus PIM forms a twisted cone as it leaves the cricoid attachment and courses laterally to attach onto the medial surface of the thyroid lamina. The Rhesus PIM does not have distinct bellies. Rather, it has two intermuscular septae dividing the muscle into three divisions, and anterior, middle and posterior. Similar to the human, the Rhesus unwinds approximately one-quarter turn, approximately 90 degrees, as it courses posteriorly. Different from the human PIM, the Rhesus PIM muscle fans out in a pinnate fashion to attach to approximately two thirds of the medial surface of the thyroid lamina, excluding the upper one third. There is no inferior border attachment. Further, the Rhesus PIM does not share any fibers with the cricothyroid muscle. The posterior extent of the PIM attachment ends before the root of the inferior thyroid horn. The anterior extent of Rhesus PIM attachment is approximately three to four millimeters posterior to the angle of the thyroid cartilage. (Figure 2 and 3). Innervations of the Rhesus PIM have not been established. Histological and histochemical studies have not been completed at this time.

4.0 Comparative analysis

Measurements from both specimen were made. Using the laryngeal lumen as a referent several dimensions of the human larynx were made. The schematic below shows the dimensions measured and the graph on the next page represents the data. It can be seen that adult human laryngeal lumen is approximately two times the size of the adult Rhesus. However, when the data are normalized, It can be seen that the Rhesus has a larger thyroid lamina and width of laminar attachment. Whereas, the human has the longer over all muscle length.

Figure 6.

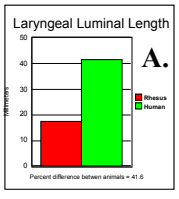


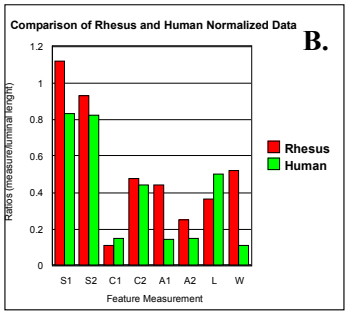
Locations of measurements from human and Rhesus larynges

Idealized skeleton

Figure 7.

Comparison Graphs
Human (*green*) and Rhesus (*red*) **(A)** laryngeal lumen lengths; **(B)** Data normalization





5. Discussion

5:1 The purpose of this study was an anatomical description of the morphology of the PIM. The PIM was found to be a flat, band-like, two belly muscle, intimately associated with both the superior cricoid arch and medial thyroid lamina. This close association may be one reason why the muscle has been over looked. Histologically and histochemically the PIM appears to be similar in every respect to the rest of the intrinsic laryngeal muscles. PIM TI/TII ratio places it at the low end of intrinsic laryngeal muscles. The presence of a muscle spindle may help to explain its function. Based on its attachments the PIM may reporting on changes in cricothyroid distance in participate vocal fold tensing.

5:2 Intrinsic laryngeal muscles are important because of their involvement with configuring the glottis for phonation and protection of the airway. The cricothyroid muscle, specifically, has been shown to have roles in fundamental frequency control (9,10,11,12,13,14) glottal shaping (4,15), and respiratory activity (16,17,18). Cricothyroid muscle morphology is assumed to be well understood as composed of broad, triangular sheets of muscle bundles divided into the pars recta (PR) and oblique (PO), and attached to the lateral surfaces of the cricoid cartilage and inferior borders and inferior horn of the thyroid cartilage (1,3). Typically, there is no mention of the PIM. A review of the literature found only three anatomical descriptions of cricothyroid muscle morphology, two of which do not mention the PIM (3,19). Physiological and pathological studies have used a variety of non-human larynges for study. These data established the action of the cricothyroid muscle to reduce the distance between the anterior cricoid and thyroid by elevating the cricoid cartilage, the so-called cricothyroid distance (11). This change in cricothyroid distance has been shown to tense the vocal folds. The PIM's anatomical position and the presence of a muscle spindle makes it uniquely suited to report on any changes in cricothyroid distance. Mammalian muscle spindles are a type of receptor used for muscle length-tension regulation (20,21).

5:3:1 Nervous innervations of skeletal muscle are related to function (22,23). Tonic muscle fibers do not normally propagate action potentials and must be stimulated to contract via a number of neuromuscular junctions, called multiterminal innervations. Multiterminal innervations have been shown to be involved in maintaining low-tension contractions lasting greater than 2.5 seconds. Long lasting, low power muscle contraction has been shown to involved in maintaining posture. Multiterminal innervations also facilitates rapid contraction and fine tension control. Greater numbers of connections between muscle and nerve produce more refined activity. Aidley (20) suggested that the intrinsic laryngeal muscles have such an arrangement. Mutliterminal innervations were not observed in the PIM. However, the absence of multiterminal innervations observed in this study may be related to the condition of the tissue at the time of harvest, the lack of specificity of the sliver stain used, or their absence anatomically.

5:3:2 Mammalian muscle can generally be divided into two groups of muscle fiber types based on histochemical properties: red and white fibers (20,24) Red muscle fibers (Type I) respond to a stimulus with a relatively slow twitch. White muscle fibers (Type II) react to a stimulus with a rapid twitch. Red muscle fibers have a more extensive blood supply than do

white fibers. Red muscle fibers are able to sustain activity for long periods, whereas white fibers produce short bursts of great tension followed by rapid onset of fatigue. Sahgal and Hast (25), while not commenting on the PIM, reported the majority of motor units of the ILM are composed of a unique fiber type that is both fast acting and fatigue resistant. The PIM appears to have a high succinate dehydrogenase content, as is found in other intrinsic laryngeal muscles. High succinate dehydrogenase content is directly correlated with the speed of a muscle.

5:3:3 The PIM may act independently of the cricothyroid proper, or it may function as part of the cricothryoid muscle group. Anatomically, it is a distinct entity. Von Mayet & Mundnich (1) and Hast (26) have argued that muscles within the thyroid cartilage are part of a phylogenetically and embryologically distinct group of muscles acting as an airway sphincter. According to Hast, intrinsic laryngeal muscles appear to develop from one of two common sets of physiologically distinct embryological muscular analage. For example, the cricothyroid muscle is derived from fourth branchial arch and inferior pharyngeal constrictor analage. Subsequently, the cricothyroid muscle develops a tensor role laryngeal physiology. All other intrinsic laryngeal muscle is derived from the sixth branchial arch primordium. Muscle originating from the sixth branchial arch is considered to be sphincteric in function (26). Hence, muscles outside the thyroid cartilage may be both phylogenetically and embryologically predisposed to act as tensors of the larynx and those within the cartilage act sphincterically. The PIM appears to be within the thyroid cartilage, but it may not play a role in laryngeal sphinctering. (Figure 7).

5:3:4 Human pars oblique (PO) and pars recta (PR) of the cricothyroid muscle have both been shown to have unimodal distribution of Type I and II muscle fibers and myoneural junctions (27,28). That is, motor endplates are diffusely distributed in the cricothyroid proper, whereas they are centrally distributed on the long axis in all other intrinsic laryngeal muscles (25). Unfortunately, this study was not able to clearly demonstrate the distribution of PIM myoneural junctions. Superficially, histological properties of the PIM place it with other intrinsic laryngeal muscles. Further histochemical study may help to establish to what group the PIM belongs.

5:3:5 It is of interest to examine the information reported here in the context of specific therapeutic considerations. For example, Botulinum toxin injection of thyroarytenoid vocalis and cricothyroid muscle is an accepted form of ameliorative therapy for forms of laryngeal dystonia known as spasmodic dysphonia. However, therapeutic effects to date have been inconsistent (2,9). Poor results may be the consequence of an incomplete understanding of laryngeal physiology. A more complete picture of laryngeal anatomy, as is being offered here, may help to resolve clinical inconsistencies.

5:3:6 Neurotization, Kojima's (30) term for transplanting a nerve-muscle pedicle in an attempt to innervate a paralyzed 2. muscle, has been demonstrated some success in mobilization of paralyzed muscles. When a nerve-muscle pedicle from the superior laryngeal nerve's external branch is surgically attached to a paralyzed muscle, some neurophysiological activity has been recorded (31). Results to date have been poor. Present techniques to re-animation of mobility impaired glottal function may not offer serious therapeutic solutions (32, 33). Having an improved understanding of the anatomy and innervations of the PIM suggests another approach. For example, it may be surgically possible to move the PIM from its thyroid lamina attachment and reattach it to the muscular process of the arytenoid.

5:3:7 Finally, laryngeal electromyography, employed clinically and in research to discern normal electrophysiology, or to record extent of neural damage and degrees of physiological dysfunction (34), has not been done on the electrophysiology PIM. Electrophysiologic investigations may enable a definition of the PIM's role in phonation and respiration. With the anatomical data presented here, investigation of PIM neuromuscular physiology in humans is possible but difficult for many reasons. And the most important being developing a recording site in the human. An animal model exists in the Rhesus macaque and will aid in the examination of PIM physiological activity. In the Rhesus monkey has a well-developed PIM, proportionally about three times the size as in the human, which may serve as an experimental model for electrophysiological studies of the muscle (Figure 8).

6. Conclusions

With this study we hoped to show the PIM to be a robust muscle in the human larynx. Its morphology is a compact two-bellied band with attachments between the anterior superio-lateral cricoid arch and the medial surface of the thyroid lamina near the inferior horn root. The PIM appears to be innervated by a small branch from the division of the internal branch of the ole in laryngeal function despite its location within the thyroid skeleton (34). In order to test this contention electrophysiological examination using a Rhesus PIM as an experimental model may prove useful.

7. Acknowledgement

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