

Comparative distribution and arrangement of Herbst corpuscles in the oropharynx of the ostrich (*Struthio camelus*) and emu (*Dromaius novaehollandiae*)

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ABSTRACT

Herbst corpuscles are widely distributed throughout the oropharynx of the ostrich and emu in contrast to the general situation in birds. Knowledge of the comparative distribution of Herbst corpuscles in the oropharynx of these two commercially important ratite species may assist in a better understanding of their feeding habits. Tissue sections representing all parts of the oropharynx of five ostrich and five emu heads collected after slaughter were prepared for light microscopy, the Herbst corpuscles counted, and the relative percentage of corpuscles calculated for defined anatomical regions. Herbst corpuscles were more widespread in the oropharynx of the emu (where they were additionally found in the tongue and laryngeal mound) than in the ostrich but were absent from the pharyngeal folds in both species. The results further indicated that Herbst corpuscles were strategically located to aid in the handling and transport of food. In this context, the high concentration of Herbst corpuscles in the prominent median palatine and ventral ridges in the ostrich denote these structures as sensory organs, namely the palatal and interramal organs. The presence of these sensory organs, coupled with the higher relative percentage of Herbst corpuscles located on the rostral oropharyngeal floor, indicate that the part of the oropharynx caudal to the mandibular and maxillary rostra forms an important sensory region in the ostrich. Additionally, species-specific concentrations of Herbst corpuscles within the oropharynx were identified which appear to assist in the accurate positioning of the tongue and laryngeal mound for cleaning the choana (internal nares) after swallowing.

KEYWORDS: *Struthio camelus*; *Dromaius novaehollandiae*; Oropharynx; Herbst corpuscle distribution; Sensory organ

INTRODUCTION

Herbst corpuscles (*Corpusculum lamellosum avium*) are lamellated sensory structures found in birds and were originally reported in the avian tongue (Herbst, 1848). They are sensitive to vibratory stimuli (Hörster, 1990; Evans and Martin, 1993) and occur at various locations throughout the body. Herbst corpuscles have been demonstrated in the avian oropharynx of a number of species (Malinovský and Zemánek, 1969; Ziswiler and Trnka, 1972; Krulis, 1978; Gottschaldt, 1985; Halata and Grim, 1993) including domestic poultry (Schildmacher, 1931; Andersen and Nafstad, 1968; Saxod, 1968; Wight et al., 1970; Hodges, 1974; Berkhoudt, 1980; Gottschaldt et al., 1982; Malinovský and Páč, 1985; Watanabe et al., 1985; Gentle and Breward, 1986) and are also present in the bill (Schildmacher, 1931;

Quilliam and Armstrong, 1963; Gottschaldt, 1974). Studies on Herbst corpuscles have focused mainly on their structure whereas reports on their distribution in the upper digestive tract have been restricted in scope. In domestic poultry, for example, their distribution has been documented in the bill of the chicken (Wight et al., 1970), bill skin of the Japanese quail (*Coturnix coturnix japonica*) (Halata and Grim, 1993) and bill skin and tongue of the mallard (Berkhoudt, 1980). In ratites, the relative distribution of Herbst corpuscles in the oropharynx of the ostrich and emu has been briefly documented (Crole and Soley, 2009a). Palmieri et al. (2002) and Tivane et al. (2006) noted a particular concentration of Herbst corpuscles in the median palatine ridge of the non-glandular region in the oropharyngeal roof of the ostrich. Guimarães et al. (2007) reported on the presence of Herbst corpuscles in the caudal third of the ostrich oropharyngeal roof. However, as

revealed by other studies in this species (Tivane et al., 2006; Tivane, 2008; Crole and Soley, 2009a), the distribution of these structures is far more widespread. Herbst corpuscles have been confirmed in the oropharynx of the emu (Crole, 2009; Crole and Soley, 2009a, b; Crole et al., 2009) and greater rhea (Feder, 1972) as well as in the bill of the kiwi (Cunningham et al., 2007).

The function of lamellated mechanoreceptors is to detect vibrational stimuli (Quilliam and Armstrong, 1963). Wight et al. (1970) suggested that the distribution of Herbst corpuscles may be linked to the structure of the bill which in turn is related to the diet of the bird, and that studying the comparative distribution of Herbst corpuscles in different types of bills may indicate their function at the specific sites where they occur. In this context, Berkhoudt (1980) reported that a correlation between mechanoreceptor distribution and feeding habits has been demonstrated in a few granivorous songbirds (Ziswiler, 1965; Ziswiler and Trnka, 1972; Krulis, 1978; Bock and Morony, 1978). Although the feeding strategy of ratites has been documented (Bonga Tomlinson, 2000; Gussekloo and Bout, 2005); future studies on the specific feeding habits of the ostrich and emu may be easier to interpret if the distribution of Herbst corpuscles is known.

Significant environmental changes may be communicated to the brain via the simultaneous stimulation of numerous single corpuscles in one location or possibly by clusters of corpuscles at different sites (Quilliam and Armstrong, 1963). In the pigeon it was found that Herbst corpuscles, concentrated in the wing, constituted a functional unit or sense organ with a high specificity to vibrational stimuli, which is likely to be involved in flight control (Hörster, 1990). Similarly, concentrations of Herbst corpuscles in specific regions of the upper digestive tract of the ostrich and emu may constitute previously unknown 'sense organs'.

A comparative study of the distribution of Herbst corpuscles within the oropharyngeal cavity of the ostrich and emu would aid in determining which regions display the greatest sensitivity to vibratory stimuli. The existing brief reports on the distribution of Herbst corpuscles in the oropharynx of these two commercially important ratites (Tivane et al., 2006; Crole and Soley, 2009a) require further examination. Such data may assist in relating the investigatory nature of these birds while foraging/feeding to structures or regions in the oropharynx which display a higher density of mechanoreceptors. This paper describes the relative distribution, arrangement and orientation of Herbst corpuscles throughout the entire oropharynx of the ostrich and emu and explores the functional significance of these structures during feeding.

MATERIALS AND METHODS

A total of 5 sub-adult ostrich and 5 sub-adult emu heads, from birds of either sex, were collected after slaughter from the Klein Karoo Ostrich Abattoir (Oudtshoorn, Western Cape Province, South Africa), Oryx Abattoir (Krugersdorp, Gauteng Province, South Africa), Emu Ranch (Rustenburg, North-West Province, South Africa) and an emu farm (Krugersdorp, Gauteng Province, South Africa). All the heads were thoroughly rinsed with running tap water to remove mucus, blood and regurgitated food. Additionally, 5 ostrich chick heads (2-4 weeks-old) and 1 emu chick head (8 weeks-old) were collected for histology of the bill. Chicks were used as the entire bill could be viewed histologically on a single slide. Ostrich chicks had been euthanized for a separate, unrelated study (protocol number 36-5-0623, Faculty of Veterinary Science, University of Pretoria) and the emu chick was euthanized due to health reasons.

Light microscopy (LM)

Five ostrich and 5 emu heads were collected as indicated above, immersion-fixed in 10% neutral-buffered formalin and transported to the Faculty of Veterinary Science, University of Pretoria. Care was taken to exclude air from the oropharynx by inserting a small block of wood between the bill tips.

The entire oropharynx of the right side (Fig. 1) was sampled to determine the distribution of Herbst corpuscles. To achieve removal of the mandible for subsequent tissue sampling, the left and right quadratomandibular joints were disarticulated and the esophagus and soft tissue incised to separate the upper and lower parts of the head. Structural features of the oropharynx were described, digitally recorded with a Canon EOS 5D digital camera (Canon, Ōita, Japan) equipped with a Canon Macro 100mm lens, and annotated. The soft interramal region was removed from the bony mandible by sharp incision following the inside mandibular edge. Due to difficulty in freeing the epithelium from the underlying bone, the rostral portion of the maxilla (Region L1 and L2 in Fig. 1a and L in Fig. 1b) (removed with a band-saw) and the mandible (regions A and B in Fig. 1) were decalcified prior to further processing for light microscopy (see below). Appropriate segments of the mucosa representing all remaining parts of the oropharynx were removed from the regions indicated in Fig. 1 and their surface dimensions measured, recorded and used to calculate the area of each specific segment in mm². Each segment was cut into smaller pieces by hand in the transverse plane at approximately 5 mm intervals. These pieces were notched to ensure that they were embedded in the correct sequence for serial sectioning, after which they were processed for light microscopy (see below). The number of

corpuscles in the segments of each sampling region was counted and the relative percentage calculated for the area of each anatomical region (see below). The median value for each anatomical region was calculated for each species and plotted on a photograph of the oropharynx to depict the relative densities of Herbst corpuscles within the various regions (Fig. 2). The upper and lower bills of the 5 ostrich and 1 emu chick were removed and decalcified (see below). The upper bill was cut into transverse-sections at approximately 5 mm intervals and the lower bill was divided into left and right halves. The right halves were transversely sectioned and the left halves longitudinally sectioned. These specimens were used to provide additional/supporting evidence on the arrangement and orientation of the Herbst corpuscles.

Decalcification of the premaxillae and mandibles took place over a period of 6 weeks in an 8% formic acid solution. The samples were placed in a fresh solution fortnightly. All tissue samples were then dehydrated through 70%, 80%, 96%, and 2X 100% ethanol and further processed through 50:50 ethanol: xylol, 2X xylol and 2X paraffin wax (60-120 minutes per step) using a Shandon model 2LE Automatic Tissue Processor (Shandon, Pittsburgh, PA, USA). Tissue samples were then imbedded manually into paraffin wax in plastic moulds. Sections were cut at 4-6 μm and stained with H&E (Bancroft and Gamble, 2002). Histological sections were viewed, features of interest described and digitally recorded using an Olympus BX63 light microscope (Olympus Corporation, Tokyo, Japan) equipped with a DP72 camera and Olympus cellSens imaging software (Olympus Corporation, Tokyo, Japan), and annotated.

Enumeration of Herbst corpuscles and statistical evaluation

The tissue strips (representing the epithelial lining and underlying connective tissue) from the slides of each region were viewed at an appropriate magnification and the number of Herbst corpuscles (n) recorded. The volumetric concentration of Herbst corpuscles was transformed to a surface concentration to enable it to be related to the surface area of the region sampled. The total surface area (y) of the strips examined for that region was calculated (total length of tissue strips in mm multiplied by the average section thickness (0.005mm) in mm^2). To proportionately relate the area of tissue sampled (y – on the microscope slide) to the total area of the region (x – Fig. 1) the equation $x \div y$ was used to obtain the factor/value (z) i.e. $x \div y = z$. The number of corpuscles (n) was then multiplied by z ($n(z)$) to obtain a total value for that region (r) ($n(z) = r$). The value of r will not reflect the actual number of Herbst corpuscles present as the calculations assume the length of each

corpuscle to be 5 μm (the thickness of the histological sections). This would have resulted in a gross inflation of the actual numbers of Herbst corpuscles present and the final values were therefore expressed as a percentage and not as a total number. All the regional values (r) were added together to obtain the grand total (t) which represented 100% of the corpuscles counted in the oropharynx of each bird. The equation $r(0.01t)$ determined the corpuscular density index (CDI) which was expressed as a percentage of corpuscles in a particular region. The CDI of the pre-defined anatomical regions was determined for each of the 5 ostrich and 5 emu specimens studied (Table 1). The median CDI values were depicted graphically on a photograph of the oropharynx of each species (Fig. 2).

The null hypothesis, that the ostrich and emu were similar (in respect of the aspects studied), was tested by a Student's t test (a 2 sample assuming unequal variances) or the Mann-Whitney Rank Sum Test (where the normality test (Shapiro-Wilk) and/or equal variance test has failed (<0.05)). Values expressed were calculated using SigmaPlot, version 12.0 (Systat Software, San Jose, CA, USA) and comprised the mean, median, standard deviation, standard error of the mean, Mann-Whitney U statistic, significance and power of the test performed with alpha. Significance was set at $p=0.05$. A value of $p<0.05$ rejected the null hypothesis and a value of $p>0.05$ accepted the null hypothesis (Table 1).

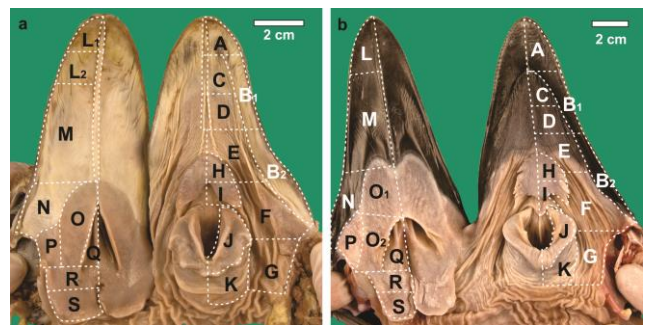


Fig. 1. Anatomical regions of the oropharynx sampled in the ostrich (a) and emu (b). Mandible (A, B1, B2), rostral keratinised floor (C, D, E), caudal non-keratinised floor (F, G), tongue body and root (H, I), arytenoid (J) and caudal (K) part of laryngeal mound, keratinised roof (L, L1, L2, M, N), non-keratinised roof (O, P), choana (Q) and rostral (R) and caudal (S) parts of the pharyngeal folds. In the ostrich the median palatine ridge is outlined and lies adjacent to regions L1, L2 and M, and the median ventral ridge is outlined and lies adjacent to regions A, C, D and the rostral part of E. Note how the median ventral ridge diverges in regions D and E and the prominent mucosal folds in regions E, F and G.

RESULTS

Regional distribution

The distribution of Herbst corpuscles, in terms of relative percentages, is presented for each anatomically defined region of the oropharynx and is detailed in Table 1 and depicted in Figure 2.

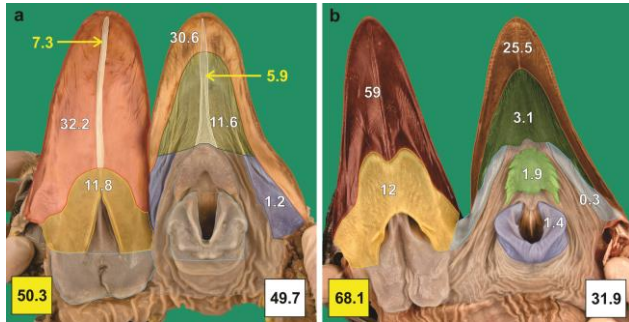


Fig. 2. Ostrich (a) and emu (b) oropharynx openly displayed to show the relative distribution of Herbst corpuscles between the two species. Numbers of Herbst corpuscles are expressed as a relative percentage (median value rounded to the first decimal place of $n=5$, see Table 1) and reflect the relative density of Herbst corpuscles in each outlined anatomical region proportional to its area (corpuscular density index). Numbers in the bottom corners show the relative percentage of Herbst corpuscles in the roof (yellow block) and floor (white block) of the oropharynx of each species. Non-shaded areas were not sampled and the grey shading represents sampled regions where corpuscles were not observed or occurred at a very low relative percentage.

The mandible - The mandible of the ostrich and emu accommodated 30.6% and 25.5% of the total percentage of Herbst corpuscles, respectively (Table 1, Fig. 2). In both species, the Herbst corpuscles in this region were concentrated in the rostrum. At the rostral extremity of the mandibular rostrum the Herbst corpuscles were located within numerous pits in the dentary bone. In the remainder of the rostrum (Region A in Fig. 1), the corpuscles formed continuous chains or sheets within the compressed connective tissue between the dentary bone and epithelium or within or near bony pits (Fig. 3) and were closely associated with nerves. A median ventral ridge displaying a high concentration of corpuscles (Fig. 4) was present on the mandibular rostrum and non-glandular region of the oropharyngeal floor (see below) in the ostrich but not in the emu. Due to the exclusive nature of the median ventral ridge, both in location and structure, it was considered to represent a regional entity with its own concentration of Herbst corpuscles. In the mandibular arms (Region B in Fig. 1) Herbst corpuscles were not as densely packed as in the rostrum and were located in the compressed connective tissue between the bone and epithelium.

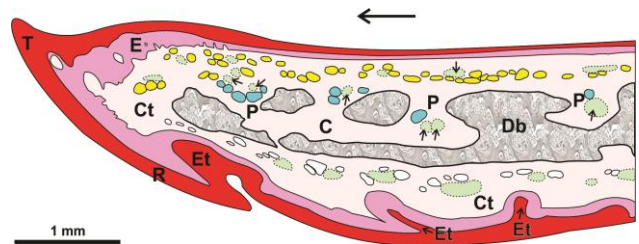


Fig. 3. Schematic representation of a longitudinal section of the mandibular rostrum in an ostrich chick. The top arrow indicates rostral and is located on the dorsal (intra-oral) surface. Note the arrangement of Herbst corpuscles (yellow shapes) which form a continuous chain in the connective tissue (Ct) between the keratinised stratified squamous epithelium (E) and the dentary bone (Db). Herbst corpuscles (blue shapes) also occur in cavities (C) within the bone and which open to the connective tissue via pits (P). Herbst corpuscles (white shapes) in the connective tissue on the ventral surface are often associated with epidermal troughs (Et). Myelinated nerves (light green shapes with dotted outline, some indicated by arrows), *Tomium* (T) and *Rhamphotheca* (R).

The rostral keratinised non-glandular oropharyngeal floor - In this region the ostrich demonstrated a significantly higher relative percentage of Herbst corpuscles (11.6%) compared to that in the emu (3.1%) (Table 1, Fig. 2). The corpuscles were located within the connective tissue approximately midway between the skeletal muscle layer and the keratinised stratified squamous epithelium. The connective tissue was not as compact in this region as in the mandible. In both species, Herbst corpuscles were concentrated within mucosal folds of this region and generally occurred in groups. A continuation of the median ventral ridge was present in the ostrich (Fig. 4), typically displaying a concentration of Herbst corpuscles. However, a comparable ridge was not present in the emu.

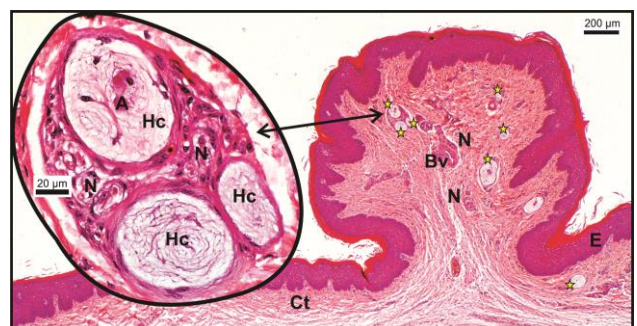


Fig. 4. Transverse section of the median ventral ridge in the ostrich (adjacent to region D in Fig. 1a). The Herbst corpuscles (Hc and stars) occur either singly or in groups (see inset) and vary in size. Keratinised stratified squamous epithelium (E), blood vessel (Bv), nerves (N) and connective tissue (Ct).

The caudal non-keratinised glandular oropharyngeal floor - Herbst corpuscles were sparsely distributed in this region (1.2% in the

ostrich and 0.3% in the emu) (Table 1) and were located mostly in the fold of mucosa housing the lateral mandibular gland (*Gl. mandibularis lateralis* (Crole and Soley, 2011)) (Fig. 2). The corpuscles were associated with the large, simple branched tubular glands forming the polystomatic glandular field of the lateral mandibular gland. In the caudal part of this region, adjacent to the laryngeal mound (Region G in Fig. 1), Herbst corpuscles were absent or extremely sparse. Where present, they occurred in the connective tissue beneath the simple tubular glands.

The tongue body and tongue root - Herbst corpuscles only occurred in the tongue body of the emu (1.9%) but not in the tongue root (Table 1, Fig. 2b). No Herbst corpuscles were located in the tongue body or root of the ostrich (Fig. 2a). Herbst corpuscles in the emu tongue body were mainly associated with the large, simple branched tubular glands present, although a few corpuscles were located within the connective tissue adjacent to the *Paraglossum* or below the surface epithelium. Herbst corpuscles in the tongue body of the emu have previously been described in detail (Crole and Soley, 2009b).

The laryngeal mound - Herbst corpuscles were only identified in the laryngeal mound of the emu, with the exception of a single Herbst corpuscle observed in one of the ostrich specimens (Table 1, Fig. 2). In the emu most of the corpuscles were found in the arytenoid part of the laryngeal mound where they were restricted to the mucosal lips lining the glottis. They were situated within the connective tissue below the *Gl. cricoarytenoidea* (Crole and Soley, 2011) and were not associated with the glands. The single Herbst corpuscle observed in the ostrich was, however, associated with a large, simple branched tubular gland of the *Gl. cricoarytenoidea*. Herbst corpuscles were only identified in the cricoid part of the laryngeal mound in one emu specimen. They were situated in the connective tissue and not associated with any specific structures.

The keratinised oropharyngeal roof - Herbst corpuscles were densely concentrated in this part of the oropharynx where they formed 32% and 59% of the total percentage of corpuscles in the ostrich and emu, respectively (Table 1, Fig. 2). Herbst corpuscles in the maxillary rostrum (Region L in Fig. 1) were similarly arranged to those in the mandibular rostrum and were densely packed, forming chains or sheets in the compressed connective tissue between the premaxilla and epithelium. They also occurred in groups in the pits of the premaxilla (Fig. 5). In the caudal parts of the keratinised oropharyngeal roof (Regions M and N in Fig. 1),

Herbst corpuscles also formed chains in the compressed connective tissue; however, in these regions not all parts of the mucosa was supported by bone. A median palatine ridge (MPR) was present in both species (Fig. 5, 6) which in the ostrich was more pronounced and contained a concentration of Herbst corpuscles (Fig. 6).

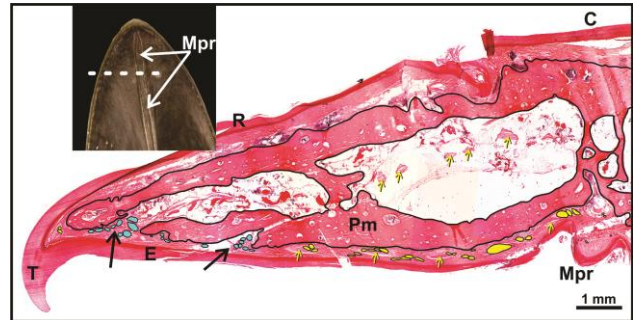


Fig. 5. A composite micrograph of a transverse section of one half of the premaxilla in the adult emu. (Region L in Fig. 1b). Inset indicates the approximate level of sectioning. Herbst corpuscles (yellow shapes) occur in chains in the connective tissue between the keratinised stratified squamous epithelium (E) and the premaxilla (Pm and outlined for clarity); they also occur (blue shapes) in pits (arrows) in the bone. *Tomium* (T), *Rhamphotheca* (R), nerves (yellow arrows), median palatine ridge (Mpr) and *Culmen* (C).

The non-keratinised oropharyngeal roof - The relative percentage of Herbst corpuscles in this part of the roof was similar in both the ostrich (11.8%) and emu (12.1%) (Table 1, Fig. 2). The corpuscles in Region O (Fig. 1) occurred throughout the underlying connective tissue and were either isolated or closely associated with the large, simple branched tubular glands (*Gl. palatina*) located in this part of the oropharynx (Crole and Soley, 2011) (Fig. 7). Isolated Herbst corpuscles were randomly positioned within the connective tissue stroma, lying beneath the surface epithelium, between adjacent glandular units, or at the base of the glands (Fig. 7). In the ostrich, a localized concentration of Herbst corpuscles was present in the mucosa along the free edge of the choana (Fig. 8). These Herbst corpuscles were not associated with glands. A localized concentration of Herbst corpuscles was present in the rictus (Region P in Fig. 1) of both birds. These corpuscles were also not associated with glands. No Herbst corpuscles were present in the mucosa within the choana (Region Q in Fig. 1) in either species.

The pharyngeal folds - Herbst corpuscles were absent from this region in both the ostrich and emu. However, in one ostrich specimen (see Table 1) a Herbst corpuscle was found in the connective tissue at the rostral limit of the region sampled. The pharyngeal folds and associated tonsils have

previously been described in both species (Crole and Soley, 2012a).

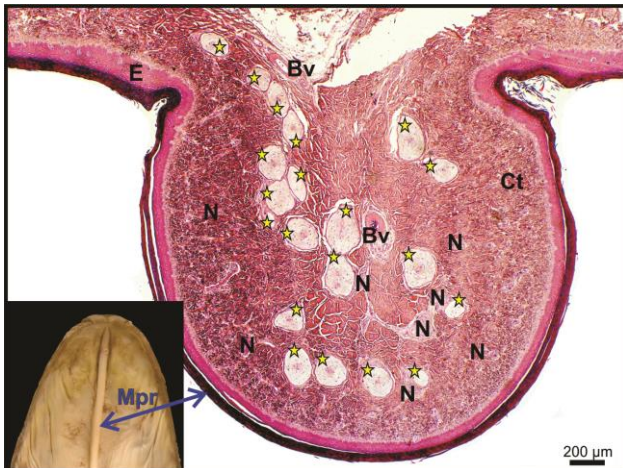


Fig. 6. Herbst corpuscles (stars) situated in the median palatine ridge (Mpr) of the keratinised oropharyngeal roof of the ostrich (bordering regions L1, L2 and M in Fig. 1a and indicated in the inset). Note the concentration of Herbst corpuscles (20 in this figure) and nerves (N) in the ridge. Connective tissue (Ct), blood vessels (Bv) and keratinised stratified squamous epithelium (E).

The median ventral ridge and median palatine ridge in the ostrich - A prominent mucosal ridge was present on the ventral midline of the oropharyngeal floor in the ostrich stretching from the mandibular rostrum to the tongue (Fig. 2), termed the median ventral ridge (MVR) (Fig. 4). In both species a ridge was also present along the midline of the keratinised oropharyngeal roof (Fig. 1, 2, 5, 6), termed the median palatine ridge (MPR); however, this structure was far more pronounced in the ostrich than the emu. Both the median ventral and palatine ridges in the ostrich contained a large concentration of Herbst corpuscles (Fig. 4, 6). A large, central artery was present at the base of the MPR and from which finer blood vessels radiated into the ridge. Accompanying the blood vessels were numerous nerves supplying the Herbst corpuscles. Although the emu displayed a small palatine ridge, there was no specific concentration of Herbst corpuscles in this structure and unlike the situation in the ostrich, in which any section displayed numerous Herbst corpuscles, in the emu many sections displayed few or none. The MVR in the ostrich was morphologically similar to the MPR and a large central artery (a branch of the sublingual artery) was also present at the base. However, as the MVR proceeded caudally it divided into two diverging ridges. The concentration of Herbst corpuscles diminished as these ridges neared the region ventral to the apex of the tongue.

Based on the Herbst corpuscle count, the ostrich displayed a greater concentration of corpuscles in the oropharynx (on average 2.5 times higher) than the emu. However, a species specific distribution of

Herbst corpuscles was apparent, with two thirds of the total percentage of corpuscles in the emu being located in the oropharyngeal roof and one third on the floor (Fig. 2). In contrast the Herbst corpuscles in the ostrich oropharynx were more evenly distributed with approximately half being present in the roof and the other half on the floor (Fig. 2).

Orientation and arrangement of Herbst corpuscles

Although the distribution of Herbst corpuscles differed in some regions between the emu and ostrich, the histological location of these mechanoreceptors and their particular arrangement was similar between the two species when viewing comparable regions. In general most Herbst corpuscles were oriented with their long axis in a rostro-caudal direction and were arranged in three specific ways: (1) those concentrated in groups clustered around nerve fibres (see Fig. 4 inset), (2) those occurring singly, or sometimes in groups, and surrounded by connective tissue (Fig. 6-8), and (3) those closely associated with large, simple branched tubular glands (Fig. 7, 8). The first arrangement was representative of Herbst corpuscles typically found beneath the intra-oral surface of the mandibular (Fig. 3) and maxillary (Fig. 5) rostra (Regions A and L in Fig. 1) as well as in the rostral portions of the median ventral (Fig. 4) and palatine (Fig. 6) ridges in the ostrich. The Herbst corpuscles were similarly arranged in the connective tissue supporting the external surfaces of the bill tip, and in the ostrich were associated with specialized epidermal troughs (Fig. 5). The second arrangement was evident at specific locations in the oropharynx reflecting single corpuscles or groups of corpuscles. They were located singly in the rostral keratinised oropharyngeal floor (Regions C-E), the tongue of the emu (Region H), the arytenoid part of the laryngeal mound in the emu (Region J) and the non-keratinised oropharyngeal roof (Fig. 7, 8) (Region O) (see also Fig. 1). Herbst corpuscles were located in groups, but without appearing to be specifically associated with a nerve, in the mandibular arms (Region B), the keratinised oropharyngeal roof (Regions M and N), the rictus (Region P), the edge of the choana in the ostrich (Fig. 8) (Region O) (see Fig. 1) and the median palatine (Fig. 6) and median ventral (Fig. 4) ridges in the ostrich. The third arrangement of Herbst corpuscles was encountered in the following glandular fields (Crole and Soley, 2011) in both species; namely, the palatine gland (Fig. 7, 8) (Region O), the oral angular gland (Region P), the lateral mandibular gland (Region F) and, in the emu only, the lingual glands (Region H).

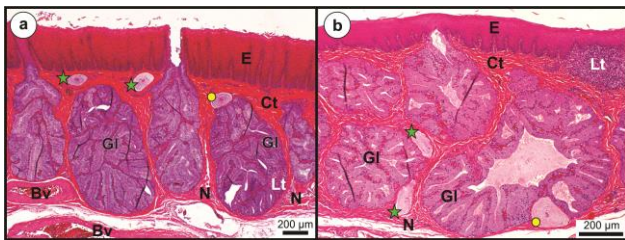


Fig. 7. Overview of the non-keratinised oropharyngeal roof in the ostrich (a) and emu (b). (Region O in Fig. 1). Some Herbst corpuscles (yellow circles) are closely associated with the glands (Gl) while others (green stars) are isolated within the connective tissue (Ct). Stratified squamous epithelium (E), lymphoid tissue (Lt), nerves (N) and blood vessels (Bv).

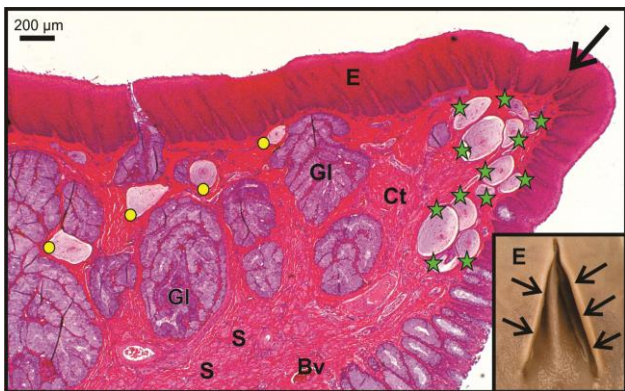


Fig. 8. Non-keratinised oropharyngeal roof in the ostrich. (Region O in Fig. 1a). Note the concentration of Herbst corpuscles (green stars) bordering the choana (arrows and inset). Smooth muscle bundles (S) are present in the connective tissue (Ct) of this region. Herbst corpuscles (yellow circles) closely associated with the glands (Gl), stratified squamous epithelium (E) and blood vessel (Bv).

DISCUSSION

Distribution of Herbst Corpuscles

Comparison between the ostrich and emu oropharynx - When comparing the two species, the ostrich displayed an even distribution of Herbst corpuscles between the oropharyngeal roof and floor, whereas two thirds of the Herbst corpuscles were present in the oropharyngeal roof and one third on the oropharyngeal floor of the emu. The present study revealed that the relative distribution of Herbst corpuscles was similar between both birds in respect of the mandible, caudal oropharyngeal floor and caudal oropharyngeal roof, whereas the ostrich displayed a higher relative distribution of Herbst corpuscles on the rostral oropharyngeal floor, and the emu a higher relative distribution in the rostral oropharyngeal roof. These results indicate that the main contributing factor to the overall difference in relative distribution of Herbst corpuscles noted in the oropharyngeal roof and floor between the ostrich (50:50) and emu (68:32) is the significantly higher relative percentage of Herbst corpuscles present on

the rostral oropharyngeal floor (including the median ventral ridge) in the ostrich. This would suggest that the rostral oropharyngeal floor is of a greater tactile importance in the ostrich than in the emu, as detailed below.

Important differences were noted regarding the distribution of Herbst corpuscles in the tongue, laryngeal mound, choana and median palatine and ventral ridges in the ostrich and emu which appeared to be linked to the specific feeding method of these birds. The ostrich and emu only use the tongue during feeding to protect the glottis (Crole and Soley, 2012c), to depress the oropharyngeal floor (Gussekkloo and Bout, 2005) and to scrape the oropharyngeal roof clear of food particles (Bonga Tomlinson, 2000). It is thus unusual that the emu tongue displays such a high relative percentage of Herbst corpuscles and the ostrich tongue none. Another peculiar point of difference between the ostrich and emu is the restriction of Herbst corpuscles to the arytenoid part of the laryngeal mound in the emu and along the edge of the choana only in the ostrich.

As noted above, the ratite tongue is used to scrape the oropharyngeal roof after swallowing (Bonga Tomlinson, 2000), which appears to be an essential process for removing any possible food items which may adhere to the region in and around the choana. The glottis of both these birds is large (Pycraft, 1900; Crole and Soley, 2012c) and any food items not removed from the vicinity of the choana could be inhaled directly into the glottis and eventually result in an inhalation pneumonia. The tongue and laryngeal mound of the emu (Crole and Soley, 2010, 2012c), ostrich (Crole and Soley, 2012c) and greater rhea (Crole and Soley, 2012b) all display their own unique adaptations for engaging with, and cleaning the choana, which would occur while the linguo-laryngeal apparatus (Crole and Soley, 2012c) is functionally in place. It has been proposed that the tongue in the greater rhea is able to be manipulated in such a manner during its retraction as to allow the two caudal lingual papillae to scrape out the heart-shaped choana (Crole and Soley, 2012b). Likewise, in the emu the centrally positioned tongue root and the small protuberances of the arytenoid part of the laryngeal mound would clean out the central groove in the choana and the infundibular cleft, and the lingual papillae of the tongue body would clean the internal nares (Crole and Soley, 2010). Additionally, in the ostrich, it would appear that the internal nares would be cleaned by the caudal laryngeal projections (personal observation). The proposed contribution of different structures in cleaning the choana in the ostrich and emu is supported by the distribution of Herbst corpuscles. In the emu, the tongue body and arytenoid part of the laryngeal mound contain Herbst corpuscles; this would provide the tactile acuity for

the emu to 'feel' whether the relevant structures were correctly positioned to scrape the choana clean. In contrast, in the ostrich, which has no corpuscles in the tongue, the Herbst corpuscles present at the edge of the choana would again afford the tactile acuity to 'feel' if the choana had been cleaned. Although Feder (1972) did not find Herbst corpuscles in the greater rhea tongue, the author admitted that their presence could not be excluded. In light of the above information, if present, they would most likely be situated in the vicinity of the caudal lingual papillae.

Comparison to the oropharynx of other birds -

The distribution of Herbst corpuscles in ratites appears to differ significantly from the situation in other birds, such as the chicken, where these structures are absent from the soft palate (caudal oropharyngeal roof), tongue and floor of the oropharynx (Winkelmann and Myers, 1961). It is difficult to draw overall comparisons between the ostrich and emu to other birds as the presence of Herbst corpuscles has only been documented for specific parts of the oropharynx in these species, for example, the rostral portion of the bill (with regards to the bill tip organ) (Goglia, 1964; Bolze, 1968; Pettigrew and Frost, 1985; Gottschaldt and Lausmann, 1974; Berkhoudt, 1976, 1980; Nebel et al., 2005; Cunningham et al., 2007, 2010a, b), the bill and/or tongue (Leitner and Roumy, 1974; Toyoshima et al., 1992), the bill and/or bill skin (Malinovský and Zemánek, 1969; Wight et al., 1970; Halata and Grim, 1993) or the oropharyngeal roof rostral to the choana (Ziswiler and Trnka, 1972). However, the distribution of Herbst corpuscles (and Grandry corpuscles) was determined in the oropharynx and bill of the mallard (Berkhoudt, 1980), although the pharyngeal folds were not included in this study. When added together, the number of Herbst corpuscles in the oropharyngeal roof was similar to that of the oropharyngeal floor in this species (Berkhoudt, 1980) as in the ostrich (see above). In common with the emu, the mallard displayed Herbst corpuscles in the tongue (Berkhoudt, 1980).

In the chicken (Wight et al., 1970) and mallard (Berkhoudt, 1980), the intra-oral surfaces of the upper bill only contained Herbst corpuscles in the soft tissue supported by bone. The distribution of corpuscles also appeared to follow the presence of underlying bony structures of the oropharyngeal roof in buntings, the pigeon, finches and swift (Ziswiler and Trnka, 1972) as well as in a number of other bird species (Schildmacher, 1931). The close association of the Herbst corpuscles with the premaxilla indicates that the presence of hard bone may increase the sensitivity of the Herbst corpuscles to vibrations (Wight et al., 1970). This contrasts sharply to the situation in the ostrich and emu where

Herbst corpuscles, in addition to those overlying bone, were also abundant in regions of the oropharynx unsupported by bone, indicating that either the Herbst corpuscles are more sensitive than those of the chicken and mallard, or that the tissue unsupported by bone is firm enough to transmit vibrations sufficiently.

Orientation of Herbst corpuscles - Most of the Herbst corpuscles observed in the present study were oriented with their long axis positioned rostro-caudally, which appears to be similar to that noted in the greater rhea (Feder, 1972). Herbst corpuscles are optimally stimulated by vibrations which are received perpendicular to the surface (Berkhoudt, 1980). Interpreted in this manner, the Herbst corpuscles in the oropharynx of the ostrich and emu would be optimally stimulated by objects moving across the surface of the oropharynx, causing vibrations perpendicular to the rostro-caudal plane, and not during the action of pecking which would transmit vibrations parallel to the long axis of the corpuscles. The orientation of the Herbst corpuscles thus seems appropriate to avoid signals generated during the forceful act of pecking, which would provide little useful tactile information.

Arrangement / grouping of Herbst corpuscles

The present study revealed that Herbst corpuscles in the oropharynx were arranged in three ways. These groupings can be placed in two broad categories; those associated with receiving tactile information (groups of corpuscles associated/not associated with a nerve as seen in arrangements 1 and 2) and those which receive stimuli from associated structures such as the mucous glands (arrangement 3). The first two arrangements have been reported in the oropharyngeal roof of the ostrich (Palmieri et al., 2002) as well as in other birds where they have been described in the bill skin of the quail (Halata and Grim, 1993), in the wing of the pigeon (Hörster, 1990) and in the bill and hard palate of the chicken (Wight et al., 1970) and finches (Genbrugge et al., 2012). The Herbst corpuscles involved in the 1st arrangement were densely packed and would function in detecting vibrational stimuli and afford a high tactile acuity. The corpuscles in the 2nd arrangement would act similarly to those above; however, the tactile acuity would depend on the density of the corpuscles (whether the corpuscles are arranged singly or in groups). Therefore, other than the bill tips (arrangement 1), specific regions of tactile acuity would be present in the median palatine and ventral ridges in the ostrich (arrangement 1), the rictus (arrangement 2) of both species and the edges of the choana (arrangement 2) in the ostrich.

There have been many reports of Herbst corpuscles being associated with specific structures,

such as feather follicles (Weir and Lunam, 2004), smooth muscle (Cobb and Bennett, 1970), the articular capsule (Halata and Munger, 1980), the crus (Zelená et al., 1997) and the bill tip organ (Bolze, 1968; Gentle and Breward, 1986; Berkhoudt, 1976). However, the association between Herbst corpuscles and glands has received little mention in the literature and has only been documented in the lacrimal gland of the chicken (Dimitrov, 2003), the salivary glands of the mallard (although sparse) (Berkhoudt, 1980) and in glands in the tongue of the emu (Crole and Soley, 2009b) and caudal oropharyngeal roof of the ostrich (Palmieri et al., 2002; Guimarães et al., 2007; Tivane, 2008). The presence of Herbst corpuscles along blood vessels suggests that these mechanoreceptors do not necessarily always serve a tactile function (Gottschaldt, 1985). Although the Herbst corpuscles associated with glands would conceivably also function in detecting tactile stimuli, their primary function may be to provide feedback on the status of the mucous salivary glands. The relationship between location and the structure and function of Herbst corpuscles has been emphasized by Gottschaldt and Lausmann (1974) who note that knowledge of the distribution of Herbst corpuscles is necessary for the interpretation and understanding of the electrophysiology and ultrastructure of these mechanoreceptors. Future functional studies will determine whether the Herbst corpuscles associated with glands serve a different function to the other Herbst corpuscles present in the oropharynx.

Functional implications during feeding

The concentration of Herbst corpuscles in the median palatine ridge (MPR) (Palmieri et al., 2002; Tivane et al., 2006; Tivane, 2008) and median ventral ridge (MVR) (Tivane, 2008) has previously been reported in the ostrich. The emu displays a less prominent MPR (Crole and Soley, 2010), and although Herbst corpuscles were present, they did not reveal a particular concentration or pattern. Additionally, the emu does not possess a corresponding MVR. A median palatine ridge has also been reported in the mallard (Berkhoudt, 1980) and concentrations of Herbst corpuscles have been identified at locations in the oropharynx of this species important during the discrimination and transport of food (Zweers et al., 1977; Berkhoudt, 1980), including the above ridge. It is clear that the ostrich must use these structures in a manner relating to the discrimination and positioning of food items, a hypothesis also forwarded by Palmieri et al. (2002). The feeding method of catch-and-throw (Gussekkloo and Bout, 2005) or cranio-inertial feeding (Bonga Tomlinson, 2000) in ratites involves only the bill tip initially holding the food. The bill tips are then opened, and the head is accelerated over the food causing the item to land in the proximal

esophagus. The mandibular rostrum is larger in the emu than in the ostrich (see Fig. 1) and is richly supplied with Herbst corpuscles. In the emu the food is most likely discriminated, held, and positioned adequately between the mandibular and maxillary rostra. In the ostrich, however, the small size of the mandibular rostrum may exclude the above actions for large food items. The presence of two ridges, richly supplied with Herbst corpuscles and which extend along the intra-oral surface of the maxillary and mandibular rostra and beyond, would implicate the involvement of these structures in the discrimination and positioning of food items before they are accelerated through the oropharynx. Thus in the ostrich, the regions caudal to the bill tip (Regions C, D, E and M in Fig. 1a) may also be indirectly involved in feeding. The positioning and potential function of these corpuscle-rich median palatine and median ventral ridges in the ostrich defines them as important sensory organs; the palatal and interramal organs, respectively.

AUTHOR CONTRIBUTIONS

The research design was the concept of MRC. MRC compiled the manuscript and JTS acted in a supervisory role on all aspects of the work and was responsible for the refinement of the manuscript. Both authors collected the specimens, discussed the results and contributed equally to the manuscript.

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TABLE 1. Comparative distribution of Herbst corpuscles (expressed as a relative percentage) in the various anatomical regions of the oropharynx of the ostrich (O) and emu (E).

n	1	2	3	4	5	Mean	MD	SD	SEM	N	E	M	p	Pa
The mandible (Regions A, B1 and B2; see Fig. 1) (Excluding the median ventral ridge in the ostrich)														
O	27.29	30.56	27.23	34.83	32.45	30.47	30.56	3.30	1.48	P	P	-	0.37	0.05
E	19.96	26.16	21.81	25.50	40.8	26.66	25.50	8.24	3.69					
The rostral keratinised non-glandular oropharyngeal floor (Regions C, D, E; see Fig. 1) (Excluding the median ventral ridge in the ostrich)														
O	12.01	11.56	10.79	13.41	10.95	11.74*	11.56	1.05	0.47	P	P	-	<0.001	1
E	3.12	4.43	5.00	1.86	2.81	3.44	3.12	1.27	0.57					
The caudal non-keratinised glandular oropharyngeal floor (Regions F, G; see Fig. 3.1)														
O	1.15	3.83	3.34	1.24	1.02	2.12	1.24	2.13	-	F	-	3	0.056	-
E	0	0	0.63	0.28	3.06	0.82	0.28	0.95	-					
The tongue body and tongue root (Regions H, I; see Fig. 1)														
O	0	0	0	0	0	0	0	-	-	F	-	0	0.008	-
E	4.29	0.92	0.81	1.85	2.37	2.17	1.85*	-	-					
The arytenoid part of the laryngeal mound (Region J; see Fig. 1)														
O	0	0.11	0	0	0	0	0	-	-	F	-	0	0.008	-
E	0.83	1.39	0.53	2.66	1.39	1.36	1.39*	-	-					
The cricoid part of the laryngeal mound (Region K; see Fig. 1)														
O	0	0	0	0	0	0	0	-	-	F	-	10	0.69	-
E	0	0	0	0	0.35	0.07	0	-	-					
The keratinised oropharyngeal roof (Regions L, M, N; see Fig. 1) (Excluding the median palatine ridge in the ostrich)														
O	29.11	34.03	32.24	32.76	30.61	31.75	32.24	1.90	-	F	-	10	0.008	-
E	58.97	61.96	59.11	54.55	38.04	54.50	58.97*	9.59	-					
The non-keratinised oropharyngeal roof (Regions O, P, Q; see Fig. 1)														
O	14.22	6.93	15.82	9.02	11.78	11.55	11.78	3.65	1.63	P	P	-	0.8	0.1
E	12.84	6.16	12.09	13.22	11.18	11.10	12.09	2.87	1.28					
The pharyngeal folds (Regions R, S; see Fig. 1)														
O	0	0	0.13	0	0	0.03	0	-	-	F	-	10	0.69	-
E	0	0	0	0	0	0	0	-	-					
The median ventral ridge (Mvr) and median palatine (Mpr) ridge in the ostrich (Regions A, C, D, L, M; see Fig. 1)														
Mvr	8.91	7.55	1.41	3.42	5.87	5.43	5.87	3.04	-	-	-	-	-	-
Mpr	7.30	5.44	9.03	5.32	7.32	6.88	7.30	1.54	-					

Median (MD), Standard Deviation (SD), Standard Error of the Mean (SEM), Normality Test (Shapiro-Wilk) (N), Equal Variance Test (E), Mann-Whitney U Statistic (M), p value (p) (bold indicates a significant difference), power of the performed test with alpha (Pa), pass (P) and fail (F). An asteriks (*) indicates a significantly higher value.