The effect of craniokinesis on the middle ear of domestic chickens (**Gallus gallus domesticus**)

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Abstract

The avian middle ear differs from that of mammalians and contains a tympanic membrane, one ossicle (bony columella and cartilaginous extracolumella), some ligaments and one muscle. The rim of the eardrum (closing the middle ear cavity) is connected to the neurocranium and, by means of a broad ligament, to the otic process of the quadrate. Due to the limited number of components in the avian middle ear, the possibilities of attenuating the conduction of sound seem to be limited to activity of the stapedius muscle. We investigate to what extent craniokinesis may impact the components of the middle ear because of the connection of the eardrum to the movable quadrate. The quadrate is a part of the beak suspension and plays an important role in craniokinesis. Micro-computed tomography was used to visualize morphology and the effect of craniokinesis on the middle ear in the domestic chicken (*Gallus gallus domesticus*). Both hens and roosters are considered because of their difference in vocalization capacity. It is hypothesized that effects, if present, of craniokinesis on the middle ear will be greater in roosters because of their louder vocalization. Maximal lower jaw depression was comparable for hens and roosters (respectively 34.1 ±2.6° and 32.7 ±2.5°). There is no overlap in ranges of maximal upper jaw elevation between the sexes (respectively 12.7 ±2.5° and 18.5 ±3.8°). Frontal rotation about the transversal quadrato-squamosal, and inward rotation about the squamosal-mandibular axes of the quadrate were both considered to be greater in roosters (respectively 15.4 ±2.8° and 11.1 ±2.5°). These quadrate rotations did not affect the columella's position and orientation. In hens, an influence of the quadrate movements on the shape of the eardrum could not be detected either, however, craniokinesis caused slight stretching of the eardrum towards the caudal rim of the otic process of the quadrate. In roosters, an inward displacement of the conical tip of the tympanic membrane of 0.378 ±0.21 mm, as result of craniokinesis, was observed. This is linked to a flattening and slackening of the eardrum. These changes most likely go along with a deformation of the extracolumella. Generally, in birds larger beak opening is related to the intensity of vocalization. The coupling between larger maximal upper jaw lifting in roosters and the slackening of the eardrum suggest the presence of a passive sound attenuation mechanism during self-vocalization.

Keywords: quadrate, craniokinesis, middle ear, chicken, micro-CT

Introduction

When vertebrates evolved from an aquatic to a terrestrial lifestyle it became advantageous a mechanical system developed to improve the transmission of sound energy in the ear by matching the acoustic impedance difference between the outside air and the fluid filled inner ear. This functional role is fulfilled by the middle ear. Without the middle ear conducting apparatus, 99.9% of the sound energy would be lost by reflection (Møller, 1974).

The mammalian middle ear consists of a tympanic membrane, three ossicles (malleus, incus and stapes), two muscles (tensor tympani muscle and stapedius muscle) and some ligaments (anterior and superior mallear ligament; posterior incudal ligament and the annular ligament). The air filled tympanic cavity containing the ossicles, muscles and ligaments is enclosed by a single bony structure, the temporal bone. This bone also supports the rim of the tympanic membrane (Ades et al., 2012, Møller, 1974, Rosowski, 1996). Effective sound transmission to the inner ear depends almost entirely on the passive acoustical (resonant) and mechanical (conductive) properties of the middle ear structures (i.e. geometry, stiffness and flexibility of the ligaments and tympanic membrane, surface ratio tympanic membrane and stapes footplate). Activity of the tensor tympani muscle and stapedius muscle can modulate these passive properties. This system has already been studied intensively. (e.g. Webster, 1966; Nuttall, 1974; Møller, 1983; Pang and Peake, 1985; Pang and Peake, 1986; Rosowski, 1996).

The avian middle ear contains a tympanic membrane; one ossicle with a bony shaft (columella) and cartilaginous, trifurcated distal end (extracolumella) (Fig.1C, green structure), with one arm (extra-stapedius) giving the tympanic membrane its conical shape; some ligaments (ascendens ligament, Platner's ligament and annular ligament) and one muscle (stapedius muscle). The middle ear cavity has an irregular shape and is bordered by bony structures of the neurocranium and quadrate. The rim of the tympanic membrane is supported by the neurocranium and rostrally by a broad ligament (see fig.1B between points A and B) that extends from the otic process of the quadrate (Fig.1B between points C and D) (Starck, 1995; Saunders et al., 2000; Smith, 1904).

In the avian middle ear, sound attenuation seems to be limited to the stapedius muscle. Primarily based on electromyograms of the stapedius muscle and associated volume change in the middle ear in chickens, Counter and Borg (1979) found the stapedius muscle to be active during vocalization, but these authors already suggested that an unknown mechanism, other than the stapedius muscle, influences the middle ear function. Starck (1995) suggested, based on anatomical research, that movement of the beak can be linked to changes in tension of the eardrum – due to the broad ligamentous connection of the tympanic membrane with the kinetic quadrate - hence effecting the hearing of birds. The present study tests this premise by quantitatively assessing the effect of craniokinesis on the middle ear.

The quadrate bone is a part of the beak suspension (Fig.1) and plays an important role in craniokinesis. A general model of upper jaw elevation has been put forward in which the quadrate rotates dorsally, rostrally and medially about the quadrato-squamosal joint. This force is transferred to the pterygoid-palatine complex and jugal, which elevates the upper jaw (Bock, 1964; Gussekloo et al., 2001; Zusi, 1984; Dawson et al., 2011). We question whether, and if so, how, these quadrate movements, which cause beak opening, may impact the conducting apparatus of the middle ear. Micro-computed tomography was used to visualize morphology. Quadrate rotations, upper jaw elevation and lower jaw depression were measured. Tympanic membrane shape analysis and columella deformation analysis were conducted to visualize the effect of craniokinesis on the middle ear in the domestic chicken (*Gallus gallus domesticus*). It is hypothesized that quadrate rotation coupled to beak opening affects the function of the sound transmitting apparatus (tympanum, extracolumella and columella) in chickens. Because of the difference in vocalization capacity between the sexes, both hens and roosters are investigated. We hypothesize that influences of craniokinesis on the middle ear will have a greater influence in roosters then in hens because of their much louder vocalization. Alarm calls in hens can reach 76 dB (Brumm et al., 2009), in roosters sexual display calls can reach 100 dB (Brackenburry, 1977; own measurements: see further).

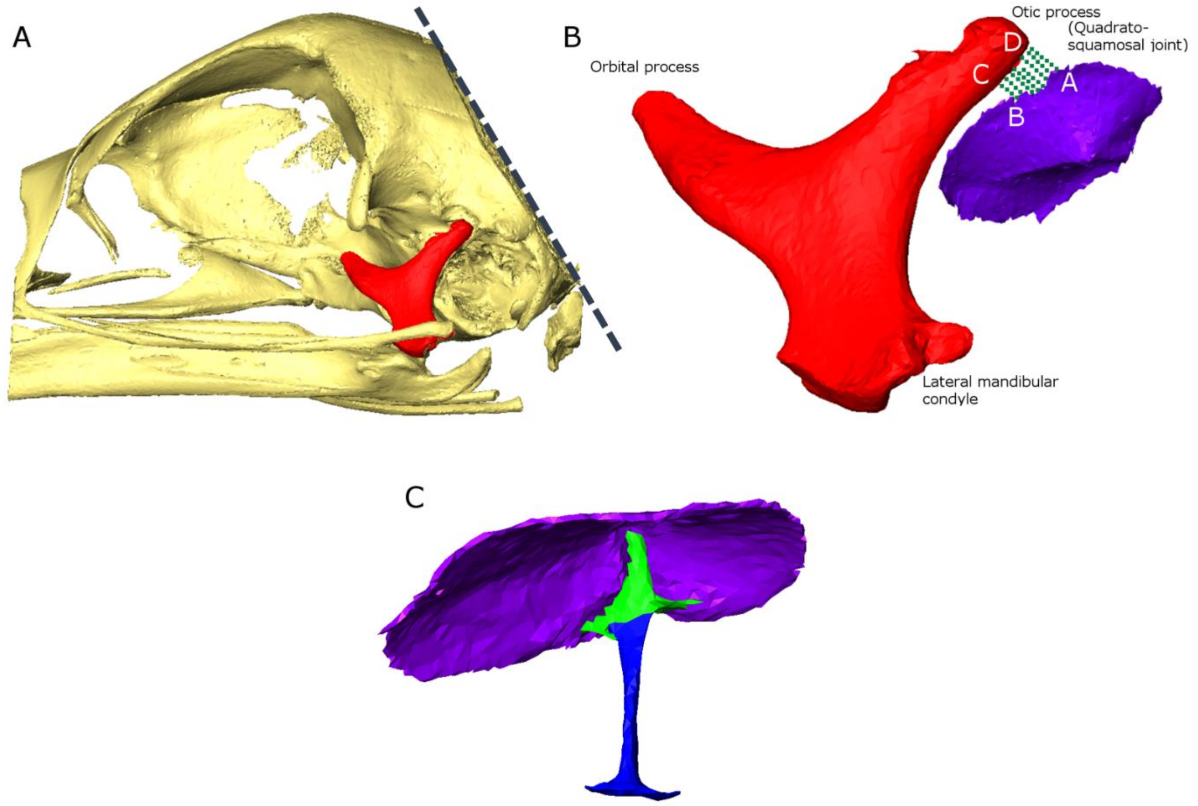

Figure 1: Chicken skull and quadrate morphology. A) Surface model of skull, lateral view. Red: quadrate; black dashed line: axis of skull removal. B) Surface model of left quadrate (red), tympanic membrane (purple) and broad ligament (green) connecting the quadrate (between C and D) and tympanic membrane (between A and B). The rest of the tympanic membrane is connected to the neurocranium. C) Middle ear components: tympanic membrane (purple); extracolumella (green) and columella (blue).

Materials and methods

Micro-CT scanning

Six heads of the domestic chicken (*Gallus gallus domesticus*), three males and three females, respectively four months and one year old, were used. The samples were stored for one day in a refrigerator and taken out the night before scanning. To exclude potential pressure fluctuations inside the middle ear as a result of the scanning procedure that could affect the tympanic membrane, the caudal part of the skull of one hen and one rooster was removed to ventilate the interaural pathway, connecting the two middle ears (Fig.1A). Removing this specific part of the skull had no influence on craniokinesis, because none of the muscles nor ligaments responsible for beak movements attach to this part of the skull (Van Den Heuvel, 1991). On these specimens, ±15 beads (glass spheres; diameter: 38 - 45 µm) were placed per tympanum on the left and right tympanic membrane. After scanning bead displacement was assessed from the difference in 3D position in the surface models. This enabled us to visualize and quantify eventual deformations of this membrane during craniokinesis. Beads displacement before and after opening of the beak could be an indication for alterations in membrane strain.

Micro-computed tomography (micro-CT) enabled us to make surface models of the specimen. The heads were scanned with the beak closed and fully opened. Care was taken not to force the beak beyond its anatomical limits but it is still possible that the manipulation range of motion do not match the behavioral range of motion. To keep the beak open during scanning, a plastic tube was placed between the upper and lower jaw. When the beak was opened, the sample was left to accommodate for 30 minutes. The scans were made by the Centre for X-ray Tomography at Ghent University with the High-Energy CT system Optimized for Research (HECTOR) (Masschaele et al., 2013). The samples were scanned over an angle of 360° in a closed container with the X-ray source set at 120 kV and 250 µA. The exposure time was set at 667 ms and the total scanning time per sample was 30 min. The reconstructed slice images had voxel sizes of 25 and 30 µm.

Segmentation, surface model

A three-dimensional image processing software package (Amira 5.4.4; 64-bit version, VSG systems) was used to segment the voxels corresponding to the quadrate, columella, tympanic membrane and the bony semi-circular canals of the inner ear. Segmentation was performed by automatic thresholding based on grey-scale values in combination with a manual correction in the three orthogonal views. The segmented outlines were smoothed and a surface model was created. The bony semi-circular canals of the inner ear were used to align the models so a comparison could be made within one head (opened and closed beak).

Measurements

Beak opening was quantified as the angle of upper jaw elevation and lower jaw depression after aligning the neurocrania of the scans with opened and closed beak. Upper jaw elevation was quantified via the four bar system formed by the quadrate (Fig.2: A); jugal bar (Fig.2: B); nasal-frontal hinge – jugal-upper jaw hinge (Fig.2: C) and the nasal-frontal hinge – quadrato-squamosal joint (Fig.2: D) (respectively crank; coupler; rocker and frame).

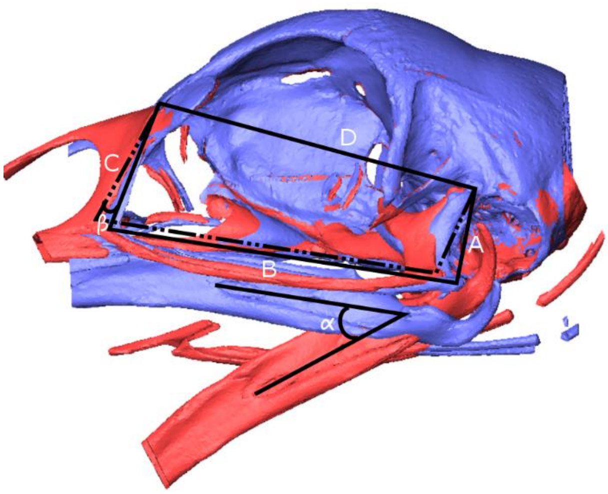

Figure 2: Morphology of hen skull with closed (purple) and open beak (red). α: lower jaw depression; β: upper jaw elevation; Position of the four bar system when the beak is closed is depicted in full lines. Position of the four bar system when the beak is opened is shown in dashed lines. A: crank; B: coupler; C: rocker; D: frame.

The morphology and dimensions of the columella, tympanic membrane and quadrates of both hens and roosters were compared to assess eventual sexual dimorphisms. Dimension differences were checked by taking the ratio of the length between the orbital process and the quadrato-squamosal joint (x) over the length between the lateral mandibular condyle and the quadrato-squamosal joint (y) (Fig.5A). The quadrate of the roosters were further downscaled and aligned with the ones of the hens to visually compare the morphology.
Rotation of the quadrate was described as a combination of a forward rotation of the lateral lower jaw condyle (Fig.1B) about the quadrato-squamosal joint (Fig.1B) and an axial rotation about its squamosal-mandibular (S-M) axis (Fig.3).

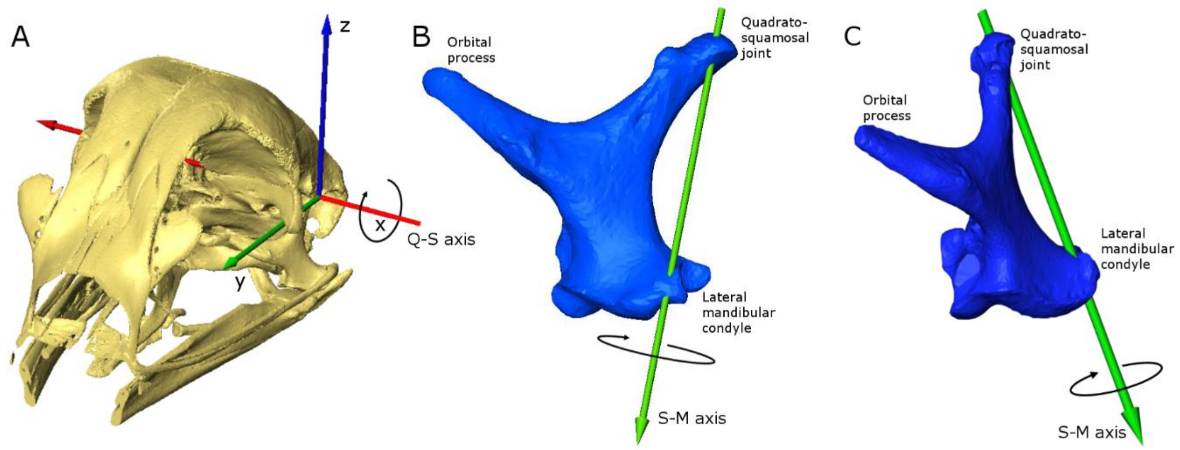

Figure 3: Axes about which the quadrate rotation angles are calculated. Directionality is indicated by the rotational arrows. A) Frontal rotation of the quadrate about the quadrato-squamosal axis (Q-S axis; red). B) Lateral view of quadrate and C) Frontal view of quadrate: axial rotation about the squamosal-mandibular axis (S-M axis; green).

The potential translation of the columella, as a result of craniokinesis, was assessed by allocating four anatomical points on the rim of the footplate. The differences between closed and open beak conditions in the position of the mean of the coordinates of these four points was calculated. Potential angular displacement of the columella was calculated as the rotation of the tip of the bony shaft about the position where the bony shaft is connected to the columella footplate.

One side of one rooster head could not be used as movement artefacts of the columella footplate during scanning occurred.

Conclusions on deformation of the tympanic membrane are based on the heads with opened interaural cavities (cf. above) only.

Behavioral recordings
For the purpose of the discussion, qualitative video recordings of pecking and crowing roosters were made of free ranging individuals and were used to assess gaping (relative to head size) of both actions. For 5 individuals, calibrated sound records of crowing were made (H2n Handy Recorder, Zoom Corporation, Tokyo; at a distance of 1m) together with the video recordings. For records for which the head was (close to) in lateral view, the reconstruction of the skull of the µCT-scanned heads with close

and maximal opened beak were superimposed on the video frames in order to estimate the degree of beak opening during crowing.

Results

Beak opening

Hens had a lower jaw depression (Fig.2: α) of 34.1 ±2.6° and roosters of 32.7 ±2.5°, the overlapping ranges are indicative for hens and roosters being indifferent in this respect. However, in upper jaw elevation (Fig.2: β) there was no overlap in ranges indicating a difference between hens and roosters (Fig.4). In hens the upper jaw was elevated 12.7 ±2.5°, whereas in roosters the upper jaw was elevated 18.5 ±3.8° (Fig.4; Table 1).

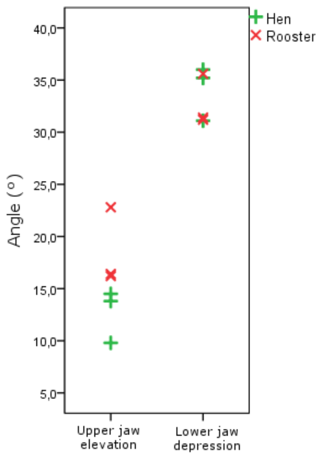

Figure 4: Measurements of upper jaw (P+M) elevation and lower jaw depression. Green: hen; Red: rooster.

Quadrate

The ratio (x/y) had a value of 1.08 ±0.06 for hens and 1.18 ±0.07 for roosters (Table 1). The small difference between these ratios, together with the comparison in figure 5, indicate that morphological differences are very small between hens and roosters. Importantly, at the otic process, where the tympanic membrane is attached no differences could be observed. Further comparison of all the quadrata showed that these differences seem to reflect inter-individual variability rather than differences between the sexes.

The frontal rotation of the quadrate about the transverse quadrato-squamosal (Q-S) axis (Fig.3A) had a magnitude of 11.1 ±2.5 for hens and 15.4 ±2.8° for roosters. The rather small overlap in ranges are indicative for roosters having a greater rotation about the Q-S axis (Fig.6, Table 1).

In hens, the quadrate rotated 3.2 ±1.2° about the squamosal-mandibular (S-M) axis (Fig.3B). In roosters this angle amounts 5.7 ±2.5°, which can be an indication that the rotation angle in roosters was greater (Fig.6, Table 1). The greatest observed quadrate displacement is situated at the point where the tympanic membrane is connected to the quadrate via the broad ligament (see point C in Fig.1B) and being equal to 0.50 ±0.07 mm in hens and 0.79 ±0.05 mm in roosters (Table 1).

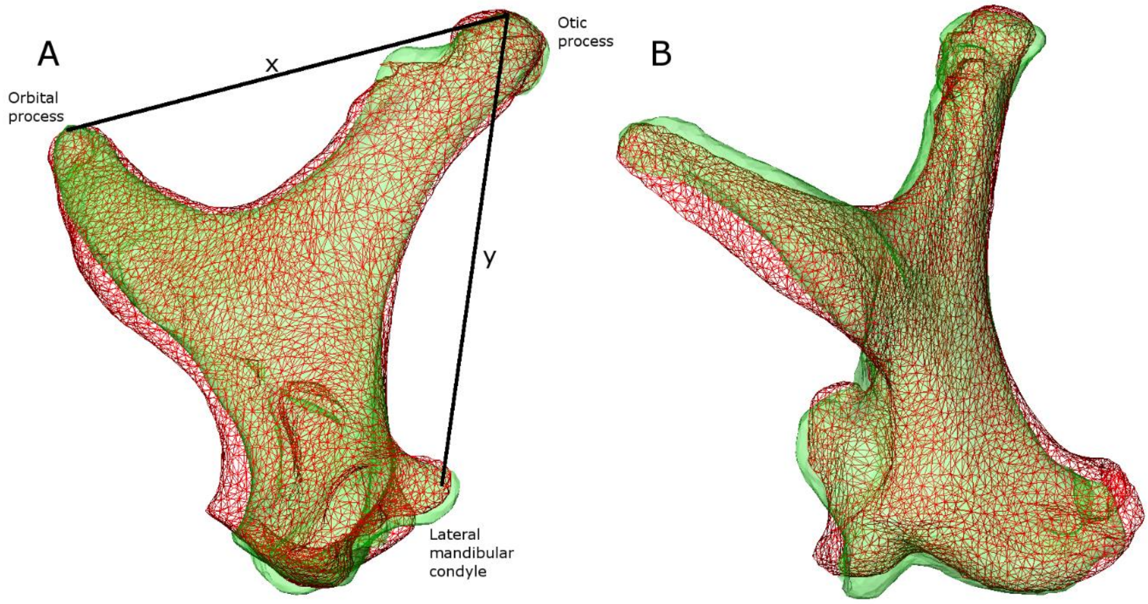

Figure 5: Surface models of the aligned left quadrates of one hen (green) and one rooster (red) after scaling. A) Lateral view; B) Frontal view.

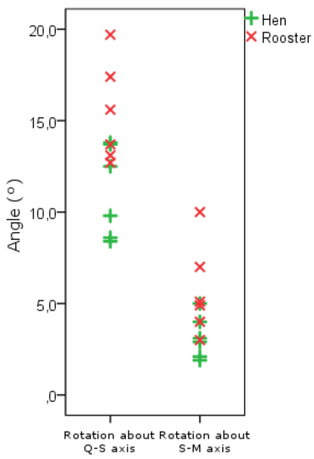

Figure 6: Measurements of frontal rotation of the quadrate about the quadrato-squamosal axis (Q-S axis) and rotation angle of quadrate about the squamosal-mandibular axis (S-M axis). Green: hen; Red: rooster.

Middle ear

A total linear columella displacement of 0.07 ±0.05 mm in hens and 0.08 ±0.02 mm in roosters was observed when the beak was opened. When linear displacement of the columella is presented as a percentage of the length of the bony shaft of the columella (respectively 2.60 ±0.05 mm in hens and 3.10 ±0.06 mm in roosters) it is noticed that linear displacement is very small, the percentages being 2.3% for hens and 2.6% for roosters. An angular displacement with a magnitude of 2.0 ±1.3° in hens and 1.3 ±0.3° in roosters was calculated (Table 1; Fig.7). Figure 7 shows that after downscaling the columella of roosters there are only minimal differences with hens.

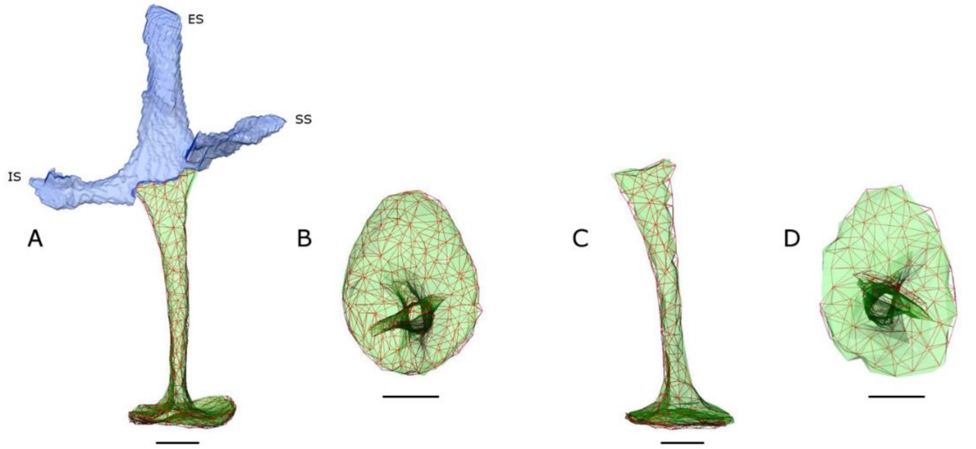

Figure 7: Position of the columella: A) lateral and B) top view of the columella of one hen and C) Lateral and D) top view of the columella of one rooster with closed beak (green) and open beak (red). Position of the extracolumella (blue) on the columella in hen with closed beak (same orientation on columella of rooster, but not shown in figure).

Figure 8A shows the effects of beak opening in hens on the tympanic membrane. No displacement of the conical tip of the eardrum - where the extra-columella pushes the eardrum outwards - was observed, in both left and right tympanic membrane (Table 1). In hens, the shape of the conus changed little, with only a small change in position of the beads (between 0.11 and 0.17 mm) towards the caudal rim of the otic process of the quadrate (Fig.9A). This suggests that beak opening, through craniokinesis, causes slight stretching of the tympanic membrane.

In roosters there is substantial inward change in position of the conical tip of the eardrum during beak opening of 0.378 ±0.21mm. This is linked to a flattening of the eardrum conus. (Table 1; Fig.8B). Figure 9B shows that direction of the biggest change in position of the beads in roosters was perpendicular at the direction of the quadrate movement. When opening the beak, a displacement of 0.145 mm and 0.115 mm on respectively the left and right tympanic membrane was calculated.

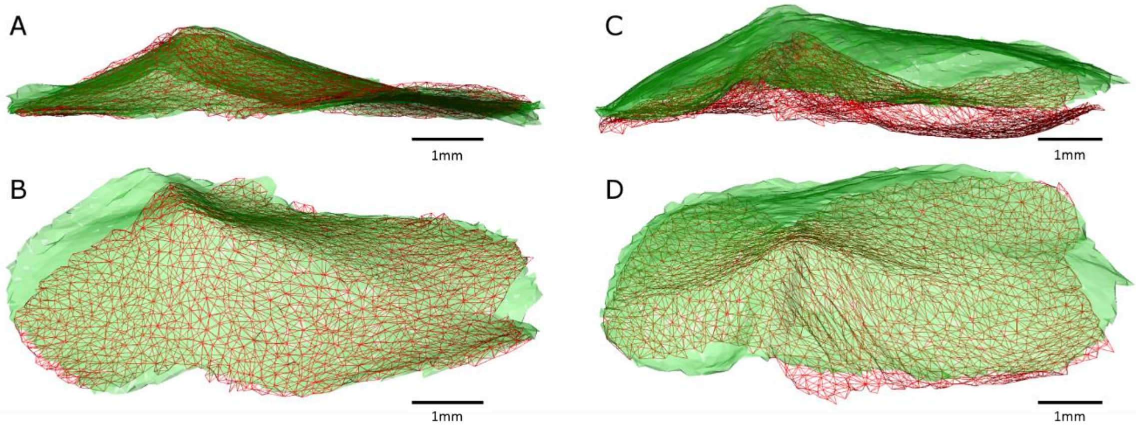

Figure 8: Color code: green: closed beak; red: maximal beak opening. A) Edge on view of the left tympanic membrane of a hen. B) Oblique view of the left tympanic membrane of a hen. C) Edge on view of the left tympanic membrane of a rooster. D) Oblique view of the left tympanic membrane of a rooster.

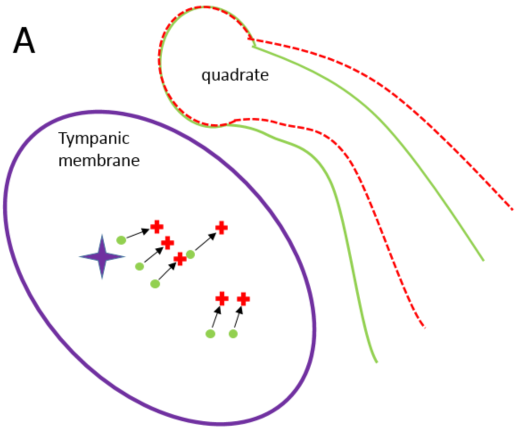

Figure 9: Schematic overview of the tympanic membrane and the otic process of the quadrate bone A) in hens and B) in roosters. Purple: tympanic membrane; green: quadrate position closed beak; red dotted line: quadrate position open beak; purple star: position of conical tip of tympanic membrane; green dot: position of beads with closed beak; red plus: position of beads with opened beak; black arrows: directions of beads (tympanic membrane) displacements (tension) (NOT magnitude of displacement).

Table 1: Overview of measured data (mean ± standard deviation) of both sexes.

		Hen	Rooster
Beak opening	Upper jaw elevation (°) (Fig.2: β)	12.7 ±2.5	18.5 ±3.8
	Lower jaw depression (°) (Fig.2: α)	34.1 ±2.6	32.7 ±2.5
Quadrate morphology	Ratio x/y (Fig.5)	1.08 ±0.06	1.18 ±0.07
Quadrate rotation	Frontal rotation angle about Q-S axis (°) (Fig.3A)	11.1 ±2.5	15.4 ±2.8
	Rotation angle about S-M axis (°) (Fig.3B)	3.2 ±1.2	5.7 ±2.5
	Quadrate displacement at point C (mm) (Fig.1B)	0.50 ±0.07	0.79 ±0.05
Middle ear	Length of columella (mm)	2.60 ±0.05	3.10 ±0.06
	Linear translation (mm)	0.07 ±0.05	0.08 ±0.02
	Angular displacement (°)	2.0 ±1.3	1.3 ±0.3
	Displacement of conical tip of TM (mm)	0.0 ±0.00	0.378 ±0.21

Discussion

The avian middle-ear conducting apparatus consists of one ossicle (the columella) composed of a bony shaft and a cartilaginous, trifurcated distal end attached to the tympanic membrane (extracolumella consisting of the supra-, infra- and extra-stapedius). There is no real joint between the bony shaft and extracolumella but great flexibility exists between the two. One muscle can be found in the avian middle ear, which is attached to the infra-stapedius and to the edge of the tympanic membrane between the infra- and extra-stapedial cartilages. Platner's ligament is connected to the transition between the extra-columella and the columella and stretches across the middle ear cavity to the

posterior face of the quadrate bone. Two other ligaments are attached to the extra- and infra-stapedius, run over the inside of the tympanic membrane and attach to the walls of the bony Eustachian groove. The tympanic membrane has a conical shape of which the tip is orientated outwards. The rim of the membrane is supported by the neurocranium and rostrally by a broad ligament that extends from the otic process of the quadrate (Smith, 1904; Saunders et al., 2000).
Counter and Borg (1979) studied the activation of the stapedius muscle in the middle ear of chickens. Electromyograms were obtained from the stapedius muscle and related to the volume changes of the middle ear. Activity of the muscle was primarily related to self-vocalization of the chickens and it was found that with the sound intensity level, the activation level of the muscle and the middle ear volume increased. However, when deactivating the stapedius muscle, volume changes were still noticeable making these authors to suggest that mechanisms other than the stapedius muscle (e.g. outer ear muscles, air pressed into the system via the Eustachian tube or deformation of the whole skull during vocalization) influence the middle ear function during vocalization. Via anatomical studies on palaeognathous birds, Starck (1995) suggested that beak opening must change tension of the eardrum, due to the connection of this membrane to the kinetic quadrate. This author postulated that this must effect the hearing of the bird. So far, no study has quantified the exact effect of craniokinesis on the middle ear in birds. The present study focused on the domestic chicken; not only because this species is widespread and well-studied (Counter and Borg, 1979; Borg et al., 1979; Borg et al., 1982; Grassi et al., 1990), but primarily because both sexes differ drastically in vocalization. Sound levels up to 100 dB - measured at 1 meter distance - are recorded during crowing (similar to the level reported by Brackenbury, 1977) and these levels are likely considerably higher at the birds external auditory canal (approximating the sound signal of crowing as an hemispheric pressure wave originating from the source, the 100 dB at 1 meter distance recalculates to an impressive 126 dB at the level of the auditory canal; Muyshondt et al., submitted). As adjustments of the middle ear mechanics in response to self-vocalization were already demonstrated in *Gallus* hens (Counter and Borg, 1979), differences between hens (which are reported to produce maximally 76 dB measured at (Brumm et al., 2009)) and roosters in the effect of the mechanical link between craniokinesis and middle ear, if present, seem therefore very plausible.

Chickens have a prokinetic skull, which means total beak opening is a combination of both upper jaw elevation, about the nasal frontal hinge, and lower jaw depression (Van Den Heuvel, 1991) (Fig.2). Due to the prokinetic skull, upper jaw elevation is caused by the rotation of the quadrate about the quadrato-squamosal joint (Van Den Heuvel, 1991; Bout and Zweers, 2001). The study of Dawson et al. (2011) also showed that in mallards rostral rotation of the quadrate is highly correlated with upper jaw elevation. Thus, solely upper jaw elevation, and not lower jaw depression, could be linked to alter middle ear mechanics via the ligamentous connection between the tympanic membrane and the caudal border of the quadrate. Our results suggest indifference in maximal lower jaw depression between hens and roosters (Fig.4). Interestingly, however, a difference in maximal upper jaw elevation between both sexes was suggested, with the greater lift in roosters (Fig.4, Table 1). As a result, roosters do show larger gaping mediated by the upper jaw. Degree of beak opening effects bird vocalizations, not only in frequency patterns (with higher gapes related to higher frequencies (Westneat et al., 1993; Podos et al., 1995; Hausberger et al., 1991)), but more importantly, also in sound amplitudes (loudness). Louder syllables are produced with greater beak opening (Goller et al., 2004; Williams, 2001). Qualitative video measurements indicated much larger gapes during crowing than during pecking the largest food items available (>3x). Protection of the inner ear against loud self-vocalization might be an issue for the roosters, because the larger gaping is likely due to larger beak opening. However, based on the superposition of the reconstructions of the skull with closed and fully opened beaks on the frames of the video sequences with the sound records, it appeared that in none of these sequences (n=9), neither upper nor lower jaw reach their maximal positions during the recorded crows. This might suggest that the behavioral range of motion (during crowing) is smaller than the anatomical range as imposed during the careful manipulation of the fresh cadaver heads used for scanning. Equally well, however, it may be that the recorded vocalizations were still submaximal. This possibility is clearly supported by the fact that the typical extended and bended neck-posture that occurs during intense crowing was never reached in the present qualitative video recordings. If true, sound levels will be even more impressive than the presently recorded 100 dB at 1m distance. Further detailed quantitative kinematical analysis coupled to sound recordings of vocalization under different natural conditions (e.g. male-male interactions, herding ….) is needed, but is beyond the scope of this anatomical study.

Given the nearly identical morphology of the quadrate in hens and roosters when scaled for neurocranial size (Fig.5, Table1) the greater maximal upper jaw rotation in roosters must be related to a greater frontal rotation of the quadrate. This goes along with also a somewhat larger outwards rotation of the caudal border of the quadrate about the S-M axis in roosters (Fig.3B, Table 1). Taken together, these quadrate rotations result in a nearly 60% larger displacement of the attachment site (measured at point C, see Fig.1; cf. table 1) of the tympanic membrane in roosters, which strongly suggests that a mechanically significant differences in effect on the middle ear between sexes must be present.

Effects of craniokinesis on the middle ear components were found in both hens and roosters. In hens change in the conical shape of the tympanic membrane could not be detected, nor in position of the conical tip (Fig.4A) However, small displacements of the beads on the tympanic membrane between the closed and open beak conditions were observed (Fig. 5A). This displacement towards the caudal rim of the eardrum where the quadrate is connected, likely indicate an increase in tension of the eardrum as the rest of the rim of the tympanic membrane is connected to the neurocranium, thus being immobile. Since the displacement of this quadrate rim is larger than the bead displacement, the broad ligament and soft tissue between quadrate and eardrum apparently absorbs part of the strain created by quadrate rotation. Any deviation from the resting tension of the tympanic membrane will result in an altered impedance (Van Dishoeck, 1941), so a shift in the sound transmission characteristics in hens as a result of craniokinesis can be expected. However, the change in tension of the tympanic membrane by craniokinesis, consequently also the premised effect on transmission, is small. To understand how tension is distributed and changes over the tympanic membrane finite element modeling is necessary for which material properties of the tympanum have to be known (e.g. thickness, elasticity, …). This, however, is beyond the scope of this study.
In roosters, craniokinesis is more pronounced and greater impacts on the tympanic membrane were observed. Our results show a clear flattening of the tympanic cone (i.e. inwards shift of the apex of the tympanic membrane). As neither linear, nor angular displacement of the columella were observed as a result of craniokinesis, this shape change of the eardrum implies a deformation of the extra-columella. Because the stapedius muscle is inactive in our specimens (cf. also Counter and Borg, 1979), tympanum movement is more readily explained by the larger quadrate movements in roosters. Moreover, pressure differentials over the membrane were excluded by experimentally opening the

interaural pathway (see materials and methods). The flattening of the cone likely results in a decreased tension in the membrane, as the membrane rim cannot move (except for the small part where it is connected to the quadrate). This is confirmed by the change in position of the beads, which move towards each other when the quadrate rotates during beak opening (Fig.9B). Relaxation of eardrum tension results in an attenuation of sound transmission, suggesting that the relatively large beak opening during a rooster's vocalization behavior (cf. above) can provide the hypothesized protective (attenuation) response via a passive kinematic chain. In hens quadrate rotations lead to an increase in tension of the tympanic membrane (see above). In roosters however a more pronounced rotation of the quadrate lead to a decrease in tension of the tympanic membrane. From the present µCT-study, however, it cannot be deduced how this more pronounced quadrate movement can lead to these opposite effects at the level of the tympanum in hens as roosters. Our samples were not stained as this may cause shrinkage and increase in stiffness (Buytaert et al., 2014). Answering the questions we pose requires to keep the natural mechanics of the tympanic membrane. Yet, not staining the specimens had as disadvantage that certain soft tissue types were not distinguishable: e.g. the transition between the tympanic membrane and the surrounding soft tissue (movements of the outer rim of the eardrum could not be visualized) and ligaments linked to the eardrum.

Conclusion

Different effects of craniokinesis on the middle ear mechanics were observed between hens and roosters. In hens, quadrate rotations when opening the beak maximally has only small effects on the tympanic membrane. In roosters, quadrate movements are more pronounced and seem to result in a decrease in tension of the eardrum, which causes a deformation of the extracolumella. Most likely, these altered mechanics are linked to larger beak opening when crowing. This difference between the sexes can be placed in a behavioral, ecological context. During pecking, beak opening remains small for both hens and roosters and sound perception (predators, destress calls, vocalizing by conspecifics) is likely not effected by the kinematic chain. For roosters, self-vocalization can be extremely loud (above 100 dB at the level of the external auditory canal). Passive damping of this intense sound level could be very helpful to protect the inner ear from being damaged.
However, more research is needed to support this hypothesis. Quantification of the strain and displacement of the tympanic membrane in function of acoustic measurement of the columella footplate response, as well as quantitative audio and video recordings of crowing roosters would be very useful in this respect.

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Author contributions

Raf Claes collected, analyzed and interpreted the data and wrote the manuscript. Pieter Muyshondt participated in data collection. Luc Van Hoorebeke and Jelle Dhaene contributed to the simulation software that helps to find optimal scan settings. Joris Dirckx and Peter Aerts were involved in setting up the study, the reporting process and in the interpretation of the data.

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