

Vibrometric studies of the middle ear of the bullfrog *Rana catesbeiana*

II. The operculum

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Accepted 15 July 2002

Summary

The operculum and stapes footplate, the two moveable elements within the oval window of the frog, have been thought to function independently. In this study, laser interferometry was used to record the vibrations of both structures in response to free-field airborne sound. Contrary to expectation, the operculum appears to be coupled to the footplate. Coupling is achieved both by means of ligaments and by a cartilaginous flange of the footplate that underlies the operculum. The stapes footplate rotates about an axis located ventrolaterally, but the axis for the operculum is dorsomedial. As a result of this unusual morphology, the opercularis muscle, which

connects the operculum and shoulder girdle, can potentially affect the movements of both the operculum and footplate. The proposed roles of the opercularis system in seismic signal detection and extratympanic sound transmission are critically reviewed in the light of this new evidence. An alternative or additional role for the opercularis system is proposed, involving the protection of the inner ear from high-amplitude displacements of the stapes footplate during breathing and vocalisation.

Key words: middle ear, bullfrog, *Rana catesbeiana*, laser Doppler vibrometry, operculum, stapes, hearing.

Introduction

As represented by that of the American bullfrog (*Rana catesbeiana*), the middle ear apparatus of frogs typically consists of a tympanic membrane connected to the oval window by means of the extrastapes and stapes. The air-filled middle ear cavity communicates with the buccal cavity via the Eustachian tube. This morphological arrangement is referred to as the tympanic system. The footplate of the stapes, seen from a dorsolateral view in Fig. 1A, consists of the proximally expanded portion of the pars media together with the pars interna. It is situated in the rostral half of the oval window in most species of frogs. The otic operculum, a roughly ovoid, cartilaginous element unique to amphibians, occupies the caudal half. The operculum articulates with the rim of the oval window around its dorsal, caudal and ventral circumference and with the stapes rostrally. A muscle, the m. opercularis, inserts on the ventral half of the operculum (Fig. 1A), which is thickened to form a muscular process. This muscle originates on the suprascapular cartilage of the shoulder girdle. The operculum and opercularis muscle together are referred to as the opercularis system. In ranid frogs, both operculum and footplate are contained within the lateral chamber, a diverticulum of the main inner ear cavity (Wever, 1985). A narrow foramen in the medial wall of the lateral chamber represents the only connection between the chamber and the inner ear proper, in which are located the receptor organs. Bolt

and Lombard (1985) believe that this foramen represents the true fenestra ovalis, but in the present study the term oval window will be applied, as above, to the much larger and more superficial opening of the skull occupied by the footplate and operculum. Fig. 1B shows a ventromedial view of the stapes footplate and operculum. A ridge on the bony pars media of the stapes, just lateral to the pars interna, forms a hinge-like articulation with the otic capsule (see Mason and Narins, 2002). The flange of the pars interna, also visible in Fig. 1B, will be described below (see Results).

The function of the operculum has intrigued biologists for many years. Hypotheses have included a kinaesthetic role (Eiselt, 1941; Baker, 1969), a means of modulating the impedance of the middle ear apparatus (Lombard and Straughan, 1974) and a protective mechanism to restrain the movement of the footplate (Wever, 1979, 1985). In recent years, a refined version of the older idea that the operculum is used in the detection of ground-borne vibrations by means of its muscular attachment to the pectoral girdle (Kingsbury and Reed, 1909) has gained prominence (Hetherington, 1985, 1987a, 1988; Hetherington et al., 1986). According to this hypothesis, low-frequency movements of the head relative to the scapula are translated by an opercularis muscle in sustained contraction into movements of the operculum relative to the skull (Hetherington, 1985, 1987a, 1988). It is proposed that the resulting pressure wave within the

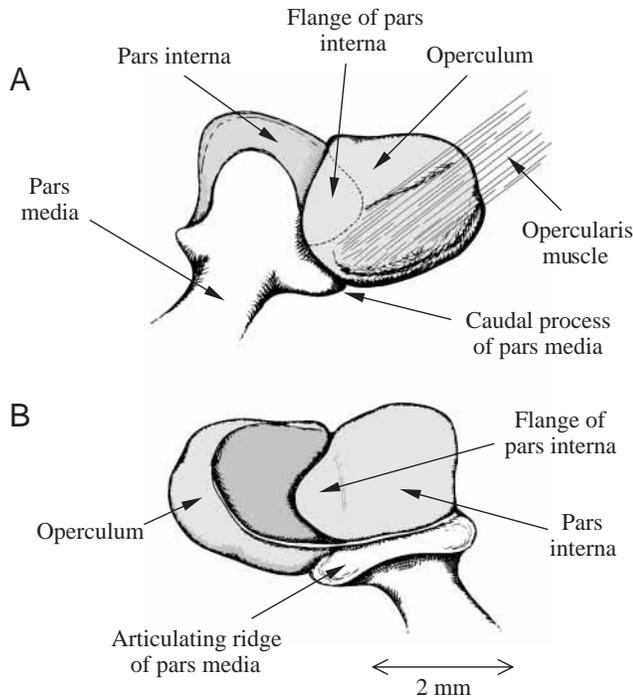


Fig. 1. The left stapes footplate and operculum of a bullfrog, seen from (A) dorsolateral and (B) ventromedial views. The stapes footplate is composed of the proximally expanded portion of the pars media together with the pars interna. Cartilage is shaded. The operculum in A is drawn as if semi-transparent to reveal the flange of the pars interna.

inner ear stimulates low-frequency, vibration-sensitive receptors such as those in the saccular or lagenar maculae (see Hetherington, 1985, 1988; Hetherington et al., 1986). As part of this hypothesis, it is argued that the tympanic system and the opercularis system are functionally independent (Hetherington et al., 1986; Jaslow et al., 1988; Hetherington, 1992).

In the present study, laser Doppler interferometry was used to record vibrations of both the operculum and stapes of the bullfrog in response to airborne sound, to examine the hypothesis that the stapes footplate and operculum are functionally independent. This is apparently the first time that direct measurements of opercular movement have been reported.

Materials and methods

Laser vibrometric measurements were performed on anaesthetised bullfrogs *Rana catesbeiana* (Shaw, 1802) 13 males and 10 females, ranging from 151 to 267 g body mass; snout-vent length (SVL) 116.7–145.1 mm. After these experiments, the animals were euthanized as described in the accompanying paper (Mason and Narins, 2002) and their middle ears were examined by anatomical dissection under light microscopy. Data from nine of these frogs were also used as part of the companion study on middle ear function (Mason and Narins, 2002). Dissections were also performed on 42 additional frogs obtained as corpses from other studies.

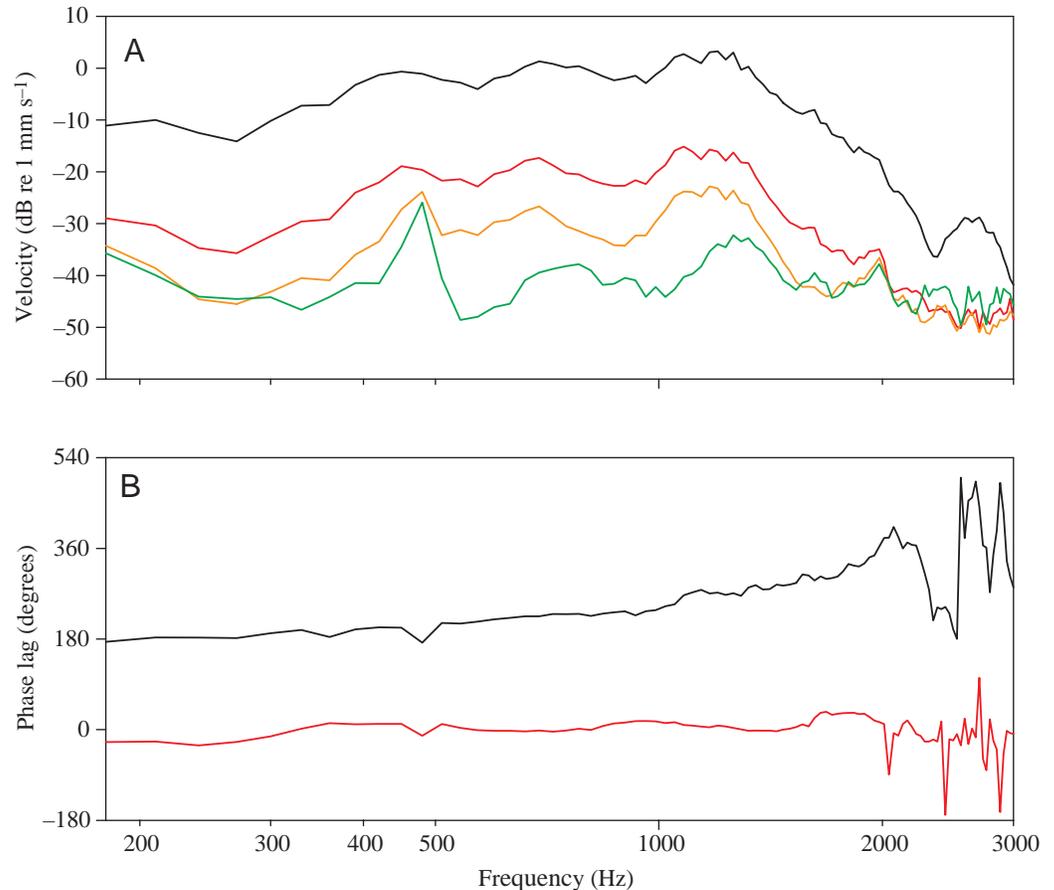
Since the methodology for the laser measurements is described in detail in the companion paper (Mason and Narins, 2002), only a brief account is presented here. The surgery used to expose the left stapes footplate and operculum from a dorsal approach allowed access to these structures without breaching the middle ear cavity and without damaging the opercularis muscle. The borders of the triangular area of the operculum visible following this surgery are delineated by the articulation with the stapes footplate rostrally, with the opercularis muscle ventrally and caudally and with the otic capsule dorsally. Up to five reflective glass beads (Polytec) were placed within this exposed region of the operculum, according to the experiment. Beads were also placed on the stapes footplate, parotic crest and tympanic membrane, as described by Mason and Narins (2002). The velocity of the parotic crest, part of the otic region of the skull of the frog, was considered to represent the velocity of the otic capsule and was used as a control for the vibrations of stapes and operculum (Mason and Narins, 2002).

Small sponge blocks were positioned under the pectoral girdle and snout of the anaesthetised frog to prop it in a sitting position. The frog was then placed on a vibration-isolated table (Backer-Loring Micro-g) within a double-walled sound-attenuating chamber (IAC 1202-A). There, it was exposed to free-field pure tones from 180 to 3 kHz, calibrated to be 90 dB SPL at the left tympanic membrane. The speaker producing the tones was resting on a separate table. A laser interferometer (Polytec OFV-303 sensor head connected to an OFV-3001 controller) was used to measure the vibration velocities of the reflective beads, which were assumed to be equal to the velocities of the structures upon which they were placed. The measurements recorded from the tympanic membrane were obtained with the laser head tilted downwards at approximately 25° to the horizontal. The velocity component measured at this angle is typically within 2 dB of the true velocity of the tympanic membrane (Mason and Narins, 2002). The vertical components of the velocities of the other structures were measured by deflecting the laser beam through a prism mounted in the barrel of a microscope (Zeiss OP-1) positioned above the frog. A custom-designed program (Acoustic Analyzer 0.20β; A. Purgue, 1999), running on an Apple Macintosh iMac computer, was used to generate and adjust the output sent to the speaker, to record the amplitude of the laser vibrometer output and to measure the phase of the returning vibrometer signal relative to the signal returning from the speaker. The sampling frequency was 44.1 kHz. Comparisons of the tympanic membrane responses obtained at the beginning and at the end of the experiments were made as controls to ensure that the response of the tympanic membrane remained approximately constant. Most runs were repeated three times and averaged; reported velocity amplitudes are peak values. Data from one male frog were rejected since the tympanic membrane was clearly drying.

Results

The result of a representative experiment in which the

Fig. 2. (A) Responses of the left tympanic membrane (black), stapes footplate (red), operculum (orange) and parotic crest (green) in a male bullfrog (SVL 123.6 mm). Note that the responses of the footplate and operculum are greater than that of the parotic crest at all but the lowest and highest frequencies. (B) Phase lags between the tympanic membrane and footplate (black) and between the footplate and operculum (red). The footplate vibrates 180° out-of-phase with the tympanic membrane at low frequencies, this phase lag increasing with frequency. However, the footplate and operculum vibrate in phase over a wide frequency range.



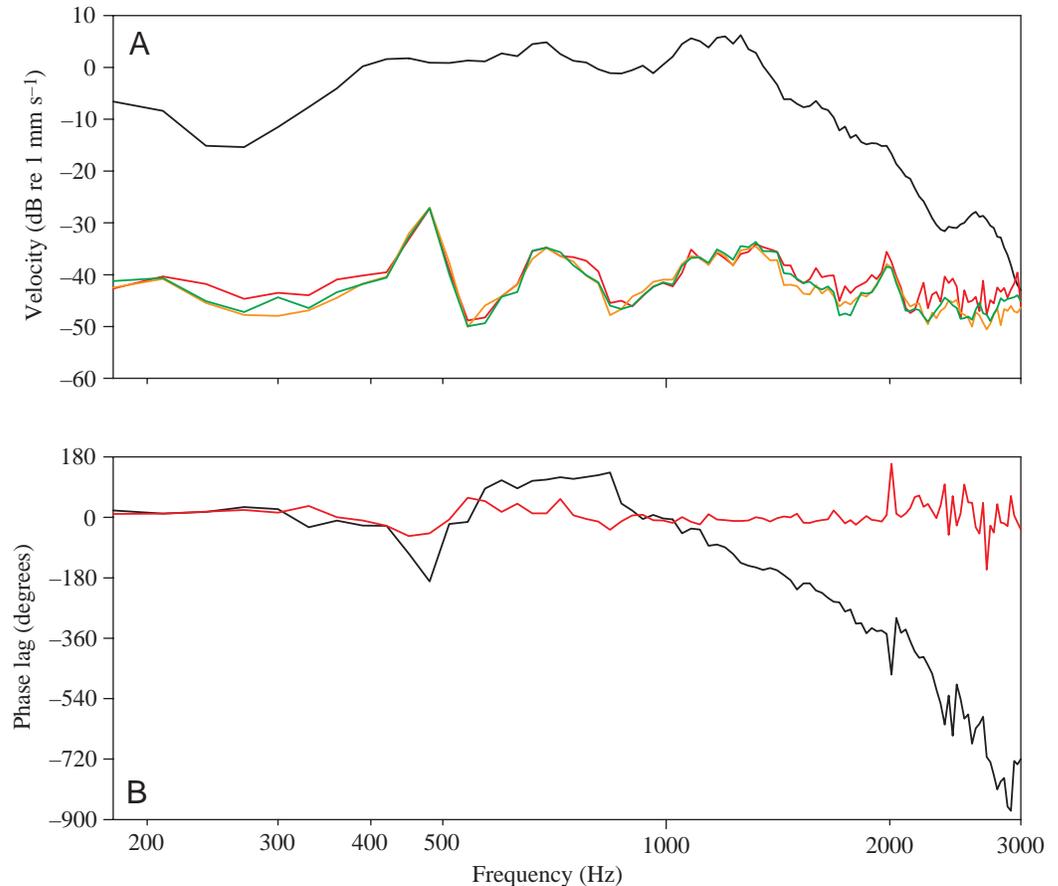
opercular bead was placed near the stapes footplate is shown in Fig. 2. The opercular velocity amplitude (orange trace) is lower than that of the footplate (red trace) at frequencies up to approximately 2 kHz, but is clearly above the 'background' response of the parotic crest (green trace) between approximately 300 and 1500 Hz (Fig. 2A). The shape of the operculum response appears to follow that of the stapes footplate and the tympanic membrane (black trace). No obvious non-linearities in the opercular response were identified at sound pressure level (SPL) values up to 100 dB (data not shown). At low frequencies, the stapes footplate velocity is 180° out-of-phase with the tympanic membrane velocity (Fig. 2B, black trace). This phase difference increases with frequency, but the stapes and operculum vibrate approximately in phase across a wide frequency range (Fig. 2B, red trace).

The similarities between the stapes footplate and operculum velocity responses suggest that the structures are coupled. However, movements of the operculum could also be caused by sound impinging on the exposed opercular surface directly or *via* vibrations of the suprascapular cartilage, conveyed to the operculum by means of the opercularis muscle (Hetherington, 1988). To examine these possibilities, the same frog as represented in Fig. 2 was removed from the sound-attenuating chamber, and the extrastapes was excised using iris scissors inserted through the wide Eustachian tube. The frog was

repositioned, and the responses of the operculum and stapes were then remeasured (Fig. 3). As expected, the velocity amplitude of the stapes footplate, uncoupled from the tympanic membrane, now matches the 'background' response of the parotic crest (Fig. 3A). The response of the operculum also falls to match that of the parotic crest. The footplate no longer shows a consistent phase relationship with the tympanic membrane, but the footplate and operculum still vibrate in phase (Fig. 3B). This experiment was performed on seven frogs. The responses of stapes footplate and operculum always dropped dramatically as a result of the excision of the extrastapes. In one animal, the responses of both structures remained clearly above the parotic crest response between 600 and 750 Hz, a frequency range at which tympanic membrane vibration was near-maximal. This could have been due to a residual mucosal connection or blood clot between the tympanic membrane and pars media, offering a means of coupling.

The measured operculum (or footplate) response is a combination of the opercular (or footplate) response relative to the otic capsule added to the 'background' vibration of the otic capsule itself. Because the relative response is presumably the effective input to the inner ear, the parotic crest response, taken to represent otic capsule vibration, was subtracted from the responses of both operculum and stapes footplate (for more details, see Mason and Narins, 2002). The operculum and

Fig. 3. (A) Responses of the same structures as represented in Fig. 2, but after the extrastapes had been severed. The tympanic membrane response is represented in black. Note that the responses of both footplate (red) and operculum (orange) have dropped to match the 'background' response of the parotic crest (green). (B) Phase lag between the tympanic membrane and the footplate (black) and between the footplate and operculum (red) in the same frog after the extrastapes had been cut. Although the footplate no longer shows the typical phase relationship with the tympanic membrane (represented in Fig. 2B), the responses of footplate and operculum remain in phase.



footplate responses presented in Figs 4 and 5 have been so adjusted.

The rotatory axes of the stapes and operculum

Fig. 4 illustrates the results of an experiment in which two beads were placed on the stapes footplate and two on the operculum, in both cases arranged with one bead medial and one lateral. The more medial bead on the stapes footplate (Fig. 4A, red trace) has a higher velocity amplitude than the more lateral bead (pink trace), consistent with a footplate rotatory axis lateral to the footplate. However, the converse is true of the operculum: the more lateral bead in this case (Fig. 4A, orange trace) has a higher velocity amplitude than the more medial bead (dark brown trace), consistent with an opercular rotatory axis medial to the operculum. At frequencies of above 2 kHz, the responses of both structures fall into the noise. The phase differences between the two footplate beads and between the two operculum beads remain very close to zero across a wide frequency range (Fig. 4B), and the relative amplitudes remain constant, indicating that these structures were not bending between the points of measurement.

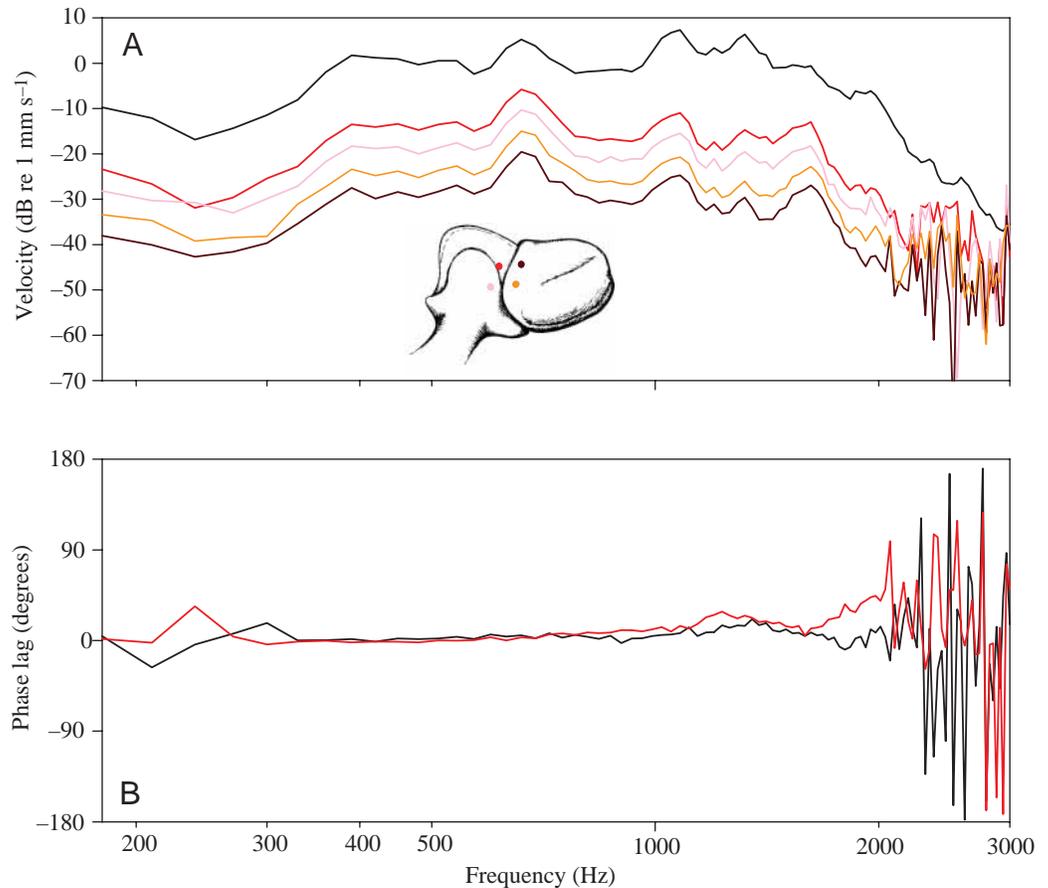
Measurements were also made from different positions arranged rostrocaudally on the operculum: a typical experiment is shown in Fig. 5, in which responses from rostral, middle and caudal opercular beads are represented as orange, red and dark brown traces, respectively. The velocity

amplitude of the operculum is similar in all three measurement positions. However, the relative amplitudes at the three measurement positions change slightly with frequency, and there are phase differences between the velocities recorded from the three positions (Fig. 5B). This suggests that the movement of the operculum is not as simple as that of a stiff plate rotating about a fixed, linear axis.

Experiments in which more than one bead was placed on the stapes were performed on five animals, with consistent results. Experiments in which more than one bead was placed on the operculum were performed on 16 frogs. In these cases, results from different animals were more variable, probably as a result of different bead positions, but showed some consistent trends: lateral positions on the operculum have a higher amplitude of vibration than medial positions; and positions near the stapes footplate vibrate in phase with the footplate and tend to have a slightly higher velocity amplitude than more caudal positions.

The opercular rotatory axis was taken to pass along the medial edge of the operculum. The angle between a line perpendicular to the axis and passing through the reflective bead on the operculum and the horizontal plane was estimated to be 20° in both sexes. The small amount of curvature of the operculum results in a slightly different angle for each bead, but this was considered negligible. The corresponding angle between the stapes footplate bead, the footplate rotatory axis

Fig. 4. (A) Responses of the tympanic membrane (black), medial footplate (red), lateral footplate (pink), medial operculum (dark brown) and lateral operculum (orange) of a male bullfrog (SVL 123.2 mm). The inset shows a representation of the left stapes and operculum, indicating the approximate positions of the reflective beads that defined the measurement positions during the experiments. There are two beads on the stapes footplate and two on the operculum. The colours of the beads, represented as dots, match the corresponding response curves. The footplate and opercular responses were adjusted for the response of the parotic crest (not shown) and for velocity component angles of 57° (footplate) and 20° (operculum). (B) Phase lag between the medial and the lateral footplate positions (red) and between the lateral and medial opercular positions (black).



and the horizontal was taken to average 57° in males and 48° in females (Mason and Narins, 2002). Since only the vertical component of vibration of the stapes and operculum was measured in this study, the measured velocity amplitudes must be divided by the cosines of these angles to estimate the true velocity at each point of measurement. This angular adjustment was taken into account in Figs 4 and 5.

Anatomical observations

In anatomical dissections, positive pressure applied to the outside of the tympanic membrane can be seen to result in downward movement of the shaft of the stapes. The footplate articulates with the otic capsule by means of a ridge of the pars media (Fig. 1B), and the footplate rotates about this articulation as the shaft moves ventrally. The resulting lever arrangement means that the footplate moves outwards as the tympanic membrane moves inwards. As the footplate moves outwards, the operculum can also be seen to move outwards, rotating about its dorsomedial border (see Fig. 6), but with a smaller amplitude than the footplate.

Coupling between the footplate and operculum in the bullfrog is the result of several anatomical features. A robust flange of the cartilaginous pars interna extends underneath the rostral third of the operculum (Fig. 1B; see also figure 1 of Lombard and Straughan, 1974). The operculum is often described as fitting into a notch in the stapes footplate (Wever,

1979, 1985; Hetherington et al., 1986; Hetherington, 1987b). This probably refers to the space between the flange and the caudal process of the pars media, which would appear as a notch in serial sections. A ligamentous band extends from the rostroventral border of the operculum between the pars interna and pars media of the stapes. Elastic tissue also extends between the pars interna and operculum at their articulation. The oval window is covered over laterally by a thick, fibrous membrane, which adheres to the internal surfaces of both the pars interna and operculum. These various ligamentous connections are strong enough that the footplate and operculum may readily be removed from the oval window still in articulation with each other.

The caudal process of the pars media of the stapes (Fig. 1) represents the point of attachment of a tough, ligamentous sheet that runs along the ventral rim of the oval window lateral to the operculum. This sheet forms an aponeurosis for the insertion of the m. levator scapulae superior, part of which is considered to be a discrete columellar muscle (Wever, 1979, 1985). The bony protuberance, being on the rotatory axis, would appear to represent the least efficient insertion point for a muscle functioning to influence the vibrations of the stapes. The intervention of the aponeurosis would also appear to transmit much of the tension applied by the muscle to the rim of the oval window rather than to the footplate. The columellar muscle described by Wever (1979, 1985) is not considered to

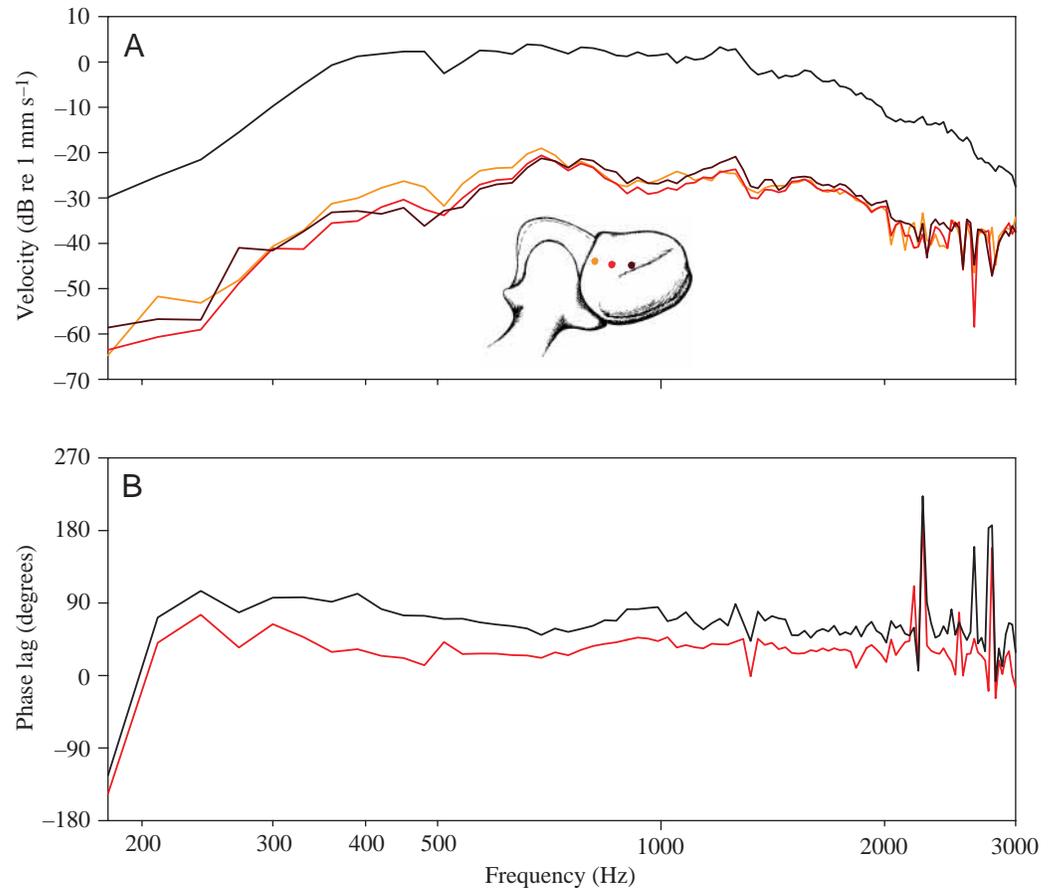


Fig. 5. (A) Responses measured from the left tympanic membrane (black) and from three positions, rostral (orange), middle (red) and caudal (dark brown), on the operculum of a female bullfrog (SVL 133.9 mm). Opercular responses have been adjusted for the response of the parotic crest (not shown) and for velocity component angles of 20°. The inset shows approximate measurement positions on the operculum. (B) Phase lags between the rostral and middle operculum (red) and between the rostral and caudal operculum (black).

exist as a discrete functional entity in most frogs (Hetherington and Lombard, 1983; Hetherington et al., 1986; Hetherington, 1987b), including the bullfrog (Hetherington and Lombard, 1983), and this view is shared by the current authors.

Discussion

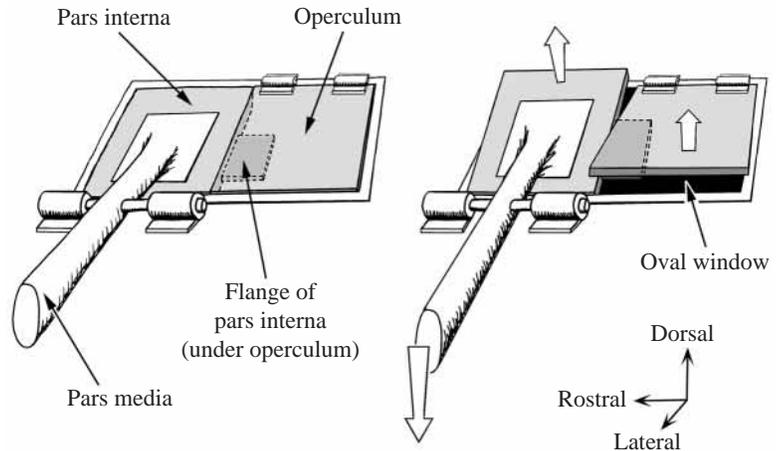
Data from both the present study and that of Jørgensen and Kannevorff (1998) are consistent with the hypothesis that the stapes footplate rotates about an axis located ventrolaterally (Bolt and Lombard, 1985), where the pars media is tightly articulated with the otic capsule. The articulation between the operculum and otic capsule is typically synchondrotic caudodorsally, with a high proportion of elastic fibres elsewhere (Hetherington et al., 1986). The hinge-like attachment of the operculum to the otic capsule has been identified by several authors on the basis of anatomy (Wever, 1979; Hetherington et al., 1986; Jaslow et al., 1988; Hetherington, 1992). Calculations made from the data obtained in the present study support the hypothesis that, in response to airborne sound, the operculum does indeed vibrate about a hinge along its dorsal border as a result of its coupling with the stapes footplate. This relationship is illustrated diagrammatically in Fig. 6.

Experimental data suggest that the operculum is tightly coupled to the footplate rostrally, although the operculum may

be forced to bend a little more caudally to accommodate footplate movement. The coupling between the stapes and operculum is contrary to the interpretation of Hetherington et al. (1986) and Jaslow et al. (1988), who state that the difference in hinge position allows these structures to move independently. Wever (1973) argues that, if the operculum were free to move in the oval window in response to acoustic pressures within the lateral chamber of the inner ear (caused by stapes footplate movement), this would represent an acoustic bypass, impairing transmission of sound to the inner ear proper. He felt that the presence of such an apparently maladaptive bypass is unlikely. The bypass hypothesis demands that the operculum move out of phase with the footplate, which is not the case. The in-phase movement of the rostral operculum and footplate is expected to minimise this shunting effect, and it also implies that the operculum will contribute to the total volume velocity at the oval window.

For each frog, the highest measured opercular velocity amplitude, always near the stapes footplate, was subtracted from the highest measured footplate amplitude. When velocity component angle was taken into account, opercular amplitudes were found to be, on average, 11.6 dB below those of the stapes footplate at frequencies below 2 kHz (21 frogs). No sex differences were apparent. The distance from this opercular measurement position to the opercular axis was typically approximately half the distance from the

Fig. 6. Diagrammatic representation of the movements of the stapes and operculum of the bullfrog at low frequencies. The operculum is portrayed as semitransparent to reveal the flange of the pars interna underneath. In this example, when the pars media is pushed down, which happens when the tympanic membrane is inflected, the footplate is moved out of the oval window. As a result of the coupling between the footplate and operculum, the operculum also moves outwards. Note that the stapes is hinged ventrolaterally but the operculum is hinged dorsomedially.



footplate measurement position to the footplate axis. This suggests that the angular velocity of the rostral operculum is, on average, 5.6 dB below that of the stapes footplate and that the coupling between the otic elements therefore operates as a lever.

To estimate the contribution of the operculum to the volume velocity at the oval window, scale diagrams of the stapes and operculum in articulation were prepared for five frogs. In each case, the position of the rotatory axis of the footplate was estimated, and the diagram was divided into 40–60 thin sections, each parallel to this axis. Assuming that the footplate rotates about this fixed axis as a stiff plate, the volume displacement of one of the thin sections will be proportional to the area of that section multiplied by its distance from the axis of rotation. The total volume displacement is approximated by the sum of the volume displacements of the sections. The volume displacement of the operculum was calculated in a similar way, except that only the area of the operculum not overlapped by the footplate flange was included. The lever effect between the footplate and operculum was taken into account by multiplying opercular volume velocity by 0.52 (–5.6 dB). Using this methodology, the operculum was calculated to contribute, on average, 19% (range 13–26%) of the total volume velocity at the oval window (volume velocity is proportional to volume displacement). Bearing in mind that the operculum seems to bend rather than to move as a rigid plate, its actual contribution will be somewhat smaller than this.

If the role of the operculum is merely to contribute a small amount to the volume velocity at the inner ear, then what would be the adaptive advantage of two elements separated by an articulation, which would inevitably introduce frictional losses into the system, over simply having a stapes footplate of greater area? The relative orientations of the opercularis muscle and opercular hinge are such that the muscle can exert maximum leverage on the operculum (Hetherington et al., 1986). This demands a dorsomedial axis of rotation, rather than ventrolateral as for the footplate, which suggests that the explanation for the complicated system might involve the opercularis muscle. Several hypotheses regarding the function

of the opercularis system are reconsidered here in the light of the new evidence from the present study.

The operculum and airborne sound transmission

Lombard and Straughan (1974) found that severing or denervating the opercularis muscle of certain hyloid and leptodactylid frogs decreased midbrain neural responses to airborne sound by up to 20–30 dB at frequencies below 1000 Hz. These authors suggested that contraction of the m. opercularis would lock the stapes and operculum together, increasing the mass and stiffness of the middle ear apparatus and decreasing the area ratio between the tympanic membrane and oval window. Wever (1979, 1985) proposed that contraction of the muscle actually decouples the stapes and operculum: the opposite of Lombard and Straughan's interpretation. The results of the present study show that the stapes and operculum of the bullfrog are coupled when the frog is anaesthetised and the m. opercularis is presumably under no more than passive tension, suggesting that Wever's hypothesis is more likely to be correct. The middle ear seems to be important in both terrestrial and underwater hearing in bullfrogs (Hetherington and Lombard, 1982), and the opercularis muscle may be in a state of sustained contraction when the frog is out of water (Hetherington and Lombard, 1983). A mechanism to change the impedance-matching properties of the middle ear between aquatic and terrestrial situations might well be advantageous (Lombard et al., 1981) and demands further investigation.

'Extratympanic' transmission of airborne sound to the inner ear is the term given to transmission by routes other than *via* the tympanic membrane (Wilczynski et al., 1987). Extratympanic transmission is more effective than tympanic transmission at frequencies below 300 Hz in *Rana pipiens* and contributes significantly to overall sound transmission at frequencies up to 1.0–1.2 kHz (Wilczynski et al., 1987). The operculum has been implicated as a possible mechanism for extratympanic transmission by means of its muscular connection to a vibrating suprascapula or through its inertia alone (Hetherington, 1985, 1987a, 1988, 1992; Jaslow et al., 1988). Wilczynski et al. (1987) anaesthetised or immobilised

their frogs, and even positioned them on their backs, in their experiments in which the effectiveness of extratympanic transmission was demonstrated, suggesting that extratympanic transmission does not depend on a tensed opercularis muscle. Indeed, microphonic response amplitude to low-frequency airborne sound decreases by only a few decibels on removal of the muscle in the bullfrog (Paton, 1971, cited in Hetherington, 1989; Hetherington, 1989); a far smaller effect than that observed by Lombard and Straughan (1974) in their hyloid and leptodactylid specimens. In the present study, the response of the operculum dropped considerably and was very close to the background skull response when the extrastapes was severed, even at low frequencies. These findings argue against an important role for the opercularis system in extratympanic airborne sound transmission in *Rana* species, at least within the frequency range considered here.

The operculum and seismic sensitivity

The hypothesis that the opercularis system is used in the detection of very low-frequency seismic vibrations was not directly addressed in this study. However, the notion that the footplate and operculum move independently within the oval window (Hetherington et al., 1986; Hetherington, 1988, 1992) needs to be revised. The foramen leading from the lateral chamber to the inner ear proper in the bullfrog is small (mean area 0.577 mm², *N*=56 left ears; this study) and is located directly underneath the operculum. This leaves no obvious opportunity for stapes and operculum vibrations to be channelled along different pathways to separate end-organs. Different inputs to the footplate and operculum will also interfere with each other as a result of their coupling. It is difficult to see how the frog would be able to identify the modality of the resulting vibrations passed to the inner ear. However, if the opercularis system works only to transmit seismic vibrations of very low frequency, whereas the tympanic system transmits higher-frequency airborne sound, it could be that the lowest frequencies reaching the inner ear of the frog are always interpreted as being of seismic origin.

The opercularis system as a protective mechanism

Wever (1979, 1985) believed that the operculum is decoupled from the stapes footplate when the opercularis muscle is contracted and the columellar muscle relaxed, the footplate then being free to move in response to airborne sound. When the opercularis muscle is relaxed and the columellar muscle contracted, Wever argued that the stapes footplate and operculum would become locked together, thus reducing stapes footplate vibration and acting as a protective mechanism, for instance when the frog was calling. Wever (1985) states that, when tension was applied to each of these muscles in turn, the electrical responses of the inner ear to airborne sound were altered in the way predicted by his hypothesis, although the results of sectioning or anaesthetising the muscles were more variable. The columellar muscle is not present in the bullfrog (Hetherington and Lombard, 1983; this study), and the opercularis muscle of anurans is primarily a

tonic muscle, adapted for slow, sustained contraction rather than for a fast, reflex response (Becker and Lombard, 1977; Hetherington, 1987a). Contraction of the opercularis muscle does not seem to affect the response of the tympanic membrane (Hetherington, 1994). A protective role for the opercularis muscle analogous to the stapedius reflex in mammals has therefore been considered unlikely (Hetherington, 1987a, 1994).

To force air into its lungs, a frog (which lacks diaphragm and ribs) increases the pressure in its buccal cavity and opens its glottis (Gans et al., 1969; de Jongh and Gans, 1969). In bullfrogs, contractions of the opercularis muscle are associated with this force-pump phase of ventilation, both when on land and when floating in water with just the nostrils above the surface (Hetherington and Lombard, 1983). Contractions are not associated with the more regular buccal cycles that exchange air within the buccal cavity only. Ventilation rate, and associated opercularis contraction, may decrease from once per second to as little as once every 10 s in a relaxed frog and may even be halted if the frog is startled (Hetherington and Lombard, 1983). However, it is argued that the association between opercularis contraction and breathing could maintain the muscle in a state of constant tension (Hetherington and Lombard, 1983; Hetherington, 1987a) and may represent an 'evolutionarily convenient' way of coupling this sustained tension to (mostly) terrestrial situations in which the feet are in contact with the substratum (Hetherington and Lombard, 1983).

The buccal cavity of the frog is in free communication with the middle ear cavity by means of the wide and permanently patent Eustachian tubes. The pressure in the middle ear cavity therefore rises with the high buccal pressure during ventilation, and the tympanic membranes bulge outwards (Hetherington and Lombard, 1983; Jørgensen et al., 1991; Narins, 1992; Narins et al., 2001). In male bullfrogs, part of the vocalisation is broadcast from the tympanic membranes, and pressure in the buccal cavity also rises during these events (Purgue, 1997). This pressure increase again results in bulging of the tympanic membranes: membrane displacements of approximately 0.2 mm have been measured, similar to the mean displacements associated with breathing (A. Purgue, unpublished observations in Purgue and Narins, 2000a). Since the stapes footplate moves 180° out of phase with the tympanic membrane at low frequencies (Jørgensen and Kannevorf, 1998; Mason and Narins, 2002; this study), the footplate will tend to be forced into the inner ear when the tympanic membrane bulges outwards (Purgue and Narins, 2000a,b) with an amplitude orders of magnitude greater than during airborne audition. Bulging may stiffen the membrane and thus reduce its response (Jaslow et al., 1988; Narins, 1992). The massive central patch of the male bullfrog tympanic membrane has a reduced vibratory response at higher frequencies due to its inertia, and this is seen as a means of decoupling the tympanic membrane vibration from the extrastapes during vocalisation (Purgue, 1997). These protective mechanisms may help to reduce the vibration

transferred to the stapes, but they do not address the quasi-static (very low frequency) displacement of the stapes caused by bulging of the tympanic membranes (onto which the higher-frequency vocalisation vibration may be superimposed). High-amplitude pressure changes within the inner ear, as caused by quasi-static displacement of middle ear ossicles, are believed to be potentially damaging (Wever, 1979; Purgue and Narins, 2000a,b). Adaptations to resist quasi-static pressure changes in the mammalian middle ear from displacing the ossicles too far may include the compliant pars flaccida of the tympanic membrane, flexible articulations between the ossicles, the middle ear muscles and certain tethering ligaments (Stenfors et al., 1979; Cancura, 1980; Marquet, 1981; Hüttenbrink, 1988; Dirckx et al., 1998). In frogs, flexibility both between the tympanic membrane and extrastapes and between the extrastapes and pars media might help to reduce the displacement of the footplate during breathing and vocalisation (Mason and Narins, 2002). In addition, a fluid bypass pathway within the inner ear of amphibians is expected to divert high-amplitude, low-frequency pressure waves away from the sensory papillae (Smith, 1968; Purgue and Narins, 2000a,b).

We propose here that the opercularis system might mediate another protective mechanism in the bullfrog, and that the link between opercularis contraction and breathing (Hetherington and Lombard, 1983) may be a direct rather than an indirect one. If operculum and footplate remain coupled, tension in the opercularis muscle could help to resist the stapes footplate from being forced into the inner ear during breathing and vocalisation. Alternatively, if the elements are decoupled during opercularis contraction, the increase in fluid pressure induced by the stapes being forced into the lateral chamber will be reduced by the operculum being both pulled outwards by the muscle and pushed outwards by the fluid pressure itself. This shunting effect, representing Wever's (1973) 'acoustic by-pass', would serve to reduce the net displacement of fluid from the lateral chamber into the inner ear proper. In dissections, pulling on the opercularis muscle moves both the operculum and stapes footplate outwards (M. J. Mason and P. M. Narins, personal observation), as predicted by the first hypothesis. Both hypotheses demand a slow contraction of the opercularis muscle concomitant with the increase in middle ear pressure, a function for which the physiological properties of the muscle (Becker and Lombard, 1977; Hetherington, 1987a) seem appropriate.

The operculum in other amphibians

Some amphibians, including urodeles and certain frogs such as *Bombina* species, lack both a tympanic membrane and a middle ear cavity but retain an opercularis system (Hetherington, 1987b, 1992; Jaslow et al., 1988; M. J. Mason and P. M. Narins, personal observation). The opercularis system in these animals clearly cannot have the same protective function proposed here for bullfrogs. In the salamander *Ambystoma tigrinum*, severing the opercularis muscle caused a small reduction in auditory responses to

airborne sound at frequencies up to 3 kHz, suggesting that the opercularis system has a role in sound reception in this species (Hetherington, 1989). However, on the basis of experiments in which tension was put on the muscle, Wever (1985) argued that the opercularis system has a protective function whereby muscular contraction reduces responses to airborne sound. The experimental evidence therefore seems inconclusive.

When the operculum of salamanders is moved inwards, perilymphatic fluid is pushed through the perilymphatic foramen from the otic capsule to the cranial cavity (Smith, 1968; Ross and Smith, 1982). Flexing the head downwards relative to the spine results in fluid movements in the opposite direction (Smith, 1968). Evidence suggests that acoustic vibrations may also pass between the ears and cranial cavity (Wever, 1978). In frogs, pressure waves conducted from the vertebral canal to the inner ear have been implicated as potentially mediating extratympanic airborne hearing (Narins et al., 1988). Since the head moves relative to the body when a bullfrog is exposed to ground vibrations (Hetherington, 1988), this could represent an alternative means of seismic detection. The opercularis system might conceivably have a protective role in reducing large pressure changes in the inner ear fluids induced by relative movement of the skull and spine during ambulatory or ventilatory movements in amphibians with or without tympanic ears.

We contend that the role or roles of the opercularis system are far from clear and are not necessarily the same in all amphibians. Direct measurements of opercular and footplate motion with the opercularis muscle in different states of contraction, and in response to seismic vibration as well as airborne sound, would be a useful direction for future study.

We wish to thank C. Lin for performing the surgery on three of the animals, R. Roverud for many helpful discussions and M. Kowalczyk for assistance with the figure preparation. This work was supported by Grant No. R01 DC00222 from the NIDCD, National Institutes of Health, to P.M.N.

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