Input-output characteristics of the tectorial membrane in the frog basilar papilla

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Abstract:
The basilar papilla (BP) in the frog inner ear is a relatively simple auditory receptor. Its hair cells are embedded in a stiff support structure, with the stereovilli connecting to a flexible tectorial membrane (TM). Acoustic energy passing the papilla presumably causes displacement of the TM, which in turn deflects the stereovilli and stimulates the hair cells.

In this paper we present optical measurements of the mechanical response of the TM to various stimulus levels. Results were obtained from three specimens (four ears). The TM response was linear for stimulus levels up to -30 dB (re. 1 μm) at the operculum. This amplitude was estimated to exceed that at which neural responses saturate. Therefore the mechanical response of the TM did not limit the maximum neural response.

The phase of the displaced area of the TM was constant across stimulus levels. Phase differences between the orthogonal spatial motion components were either close to either 0° or 180°. These findings were consistent with a TM motion along the flat epithelium surface.

5.1 Introduction

Due to its relatively simple anatomy and functionality, the anuran ear provides the opportunity to study fundamental properties of a vertebrate hearing organ. Like in other vertebrates, the anuran inner ear consists of two intertwined membranous labyrinths, the perilymphatic and the endolymphatic space. The sensory organs of the inner ear reside in the endolymphatic labyrinth. In the frog, there are two auditory end organs: the amphibian papilla (AP) and the BP. Both the AP and the BP have hair cells as their mechano-electrical transducers. The frog is unique in that these hair cells are set in limbic tissue (Wever 1985), and do not sit on a flexible basilar membrane. In both papillae, the hair bundles protrude into the endolymphatic space and connect to an overlying TM.

The frog’s auditory-frequency range is divided between the AP and the BP. The AP covers the lower portion, with the characteristic frequencies (CFs) of the afferents ranging from approximately 0.1 kHz to 1.4 kHz, where the upper limit is species dependent (Ronken 1991). The BP functions as the high-frequency detector with CFs ranging from 1.2 kHz to 4 kHz across species (Ronken 1991).

In this paper, we discuss experiments on the BP, exclusively. The epithelium of the BP is located in an approximately cylindrical lumen (fig. 5.1), which opens into the sacular space on its lateral end. At the medial end, the lumen terminates at the contact membrane (CM) which forms the separation between the endolymphatic and perilymphatic labyrinths.

The number of hair cells is about 60 in the bullfrog (Frishkopf and Flock 1974), and about 75 in the northern leopard frog (personal observation; chapter 3). In ranid species, the hair cell orientation is uniform: the hair cell’s excitatory direction points from the sacculus to the CM (Lewis and Narins 1999; chapter 3). There is no known electrical tuning (Smotherman and Narins 1999b). The afferent nerve fibers connecting to the BP have very similar tuning curves within an individual animal, and lower tuning sharpness than afferents from other tectorial hearing organs in the same frequency range (Ronken 1990; Evans 1975b). Thus, the BP is broadly tuned to a single CF. However, this frequency may vary between individuals.
Figure 5.1: Schematics of the frog inner ear. a: Schematic cross section of the frog ear (adapted from Wever 1985). On the right, the air-filled middle ear is displayed with the tympanic membrane and the columella. On the left, the fluid-filled inner ear is shown, with the perilymphatic fluid in white and the endolymphatic fluid in gray. The tectorial membranes of both the AP and the BP are drawn in their respective lumina. The oval window is covered by the footplate (F) of the columella, and the operculum (Op). b: Schematic of the dissected inner ear and its orientation in the experimental setup. The preparation is rotated approximately 90° relative to panel a. The arrow indicates the microscope’s viewing direction; the dashed line indicates the line of sight. The stimulator (S), placed against the operculum, is indicated on the left. The dashed gray outline indicates the area detailed in panel c. c: Cross-section of the frog’s BP anatomy (based on Frishkopf and Flock 1974; bullfrog). The arrow again indicates the viewing direction in our setup. d: Schematic of the BP as seen through the CM. e: Measurement image of the BP as seen through the microscope. The vectors to the lower right in panels b through e indicate the orientation of the panels relative to the coordinates used in data-analysis. Labels: AP - amphibian papilla, BP - basilar papilla, CM - contact membrane, E - endolymph, Ep - epithelium, N - nerve fibers, O - microscope objective, Op - operculum, P - perilymph, S - stimulator, Sac - sacculus, TM - tectorial membrane.
of a particular species. Across species studied, it ranges from about 1.2 kHz in the bullfrog, *R. catesbeiana* (Shofner and Feng 1981), to 3.9 kHz in the Puerto Rican tree frog, *Eleuthero- dactylus coqui* (Narins and Capranica 1980; reviewed in Ronken 1991 and Schoffelen et al. 2008, chapter 2). The BP’s neural thresholds are approximately 60 dB SPL, in the northern leopard frog (Ronken 1990), and thus relatively high compared to other vertebrate hearing organs.

In the northern leopard frog, the TM response is mechanically tuned to a frequency near 2.0 kHz (Schoffelen et al. 2009b, chapter 4). In an isolated preparation, the tuning quality, $Q_{10dB}$, of the TM response corresponds to that of the auditory nerve fibers, and the TM’s best frequency corresponds to the afferent nerve fibers’ CFs (Ronken 1991; Schoffelen et al. 2009b, chapter 4). Therefore, the TM’s mechanical tuning is the basis of the tuning of the BP (Schoffelen et al. 2009b, chapter 4).

The neural responses of the BP’s afferent fibers saturate at stimulus levels not far above the threshold; maximum onset spike rates occur at stimuli between 80 and 90 dB SPL, while sustained rates achieve their maximum at a stimulus level approximately 5 dB below that (Ronken 1990). We investigated to what extent the non-linear input/output (I/O) characteristics of the neural responses are based on a non-linearity in the TM response. Our results showed that in our isolated preparation the amplitude of the TM depends linearly on the input amplitude at the oval window, and that the TM response saturates at stimulus levels exceeding those needed to achieve saturation in the neural signal.

### 5.2 Materials and methods

We used four ears from three northern leopard frogs (two males, one female, m = (58±9) g), *R. pipiens pipiens*, in our experiments. The animals were obtained from a commercial supplier (Charles D. Sullivan Co. Inc., Nashville (TN), USA via Exoterra Schaudi GmbH, Holzheim, Germany), and housed at the University of Groningen laboratory-animal facilities. The experiments were approved by the local Institutional Animal Care and Use Committee.

The experimental setup, and surgical and experimental procedures corresponded closely to those detailed by Schoffelen et al. (2009b, chapter 4). The setup was built to record the mechanical response of the tectorial membrane in the frog inner ear. It consisted of a trifocal microscope (BX51WI, Olympus Corporation, Japan) with 5× (NA=0.10) and 40× (NA=0.80) objectives. The microscope was mounted on a vibration-isolating table in a darkened room. The focal plane of the 40x objective could be controlled remotely through a piezo-positioner (P-725.4CL, Physik Instrumente GmbH & Co., Karlsruhe, Germany). The microscope was equipped with a scientific-grade, monochrome digital camera (1412TE Cooler Camera, DVC Company, Austin, TX, USA), mounted on 0.5x video adapter. Its detector was CCD chip containing 1392x1040 12-bit pixels (8.9 mm x 6.7 mm). A green Luxeon power LED (Lumileds Lighting, San Jose (CA), USA) provided stroboscopic illumination. A closed-loop piezo-actuator (P-843.10, Physik Instrumente) with a custom needle was used to stimulate the inner ear.

A LabView (v8.2, National Instruments, Austin, TX, USA) computer program controlled the stimulus presentation, the stroboscopic illumination, the positioning of the objective lens, and the digital camera. Stimulus attenuation was controlled manually with an attenuator (Hewlett-Packard Co., Palo Alto, CA, USA) placed between the computer output and the stimulator’s amplifier. The applied stimulus amplitude was recorded from the piezo-
actuator’s feedback system.

At the start of the experiment, an animal was killed with a double-pith procedure. The ears were excised and kept in amphibian ringer solution (Carolina Biological Supply Company, Burlington, NC, USA). One of the ears was chosen for immediate use, while the other one was stored at approximately 5°C, for use later on the same day.

The perilymphatic space of the ear to be used was opened at the round window. This gave us a line of sight into the BP’s lumen through the contact membrane (see fig. 5.1). A perspex cylinder was glued onto the operculum to facilitate the application of the stimulation device. The preparation was positioned under the microscope and clamped into place. The needle of the piezo actuator was carefully positioned against the perspex cylinder. We adapted the camera’s orientation to optimize the image on the CCD, and determined a combination of LED driving voltage and illumination time such that the exposure during stroboscopic illumination would fill the CCD up to between 50% and 75% of its capacity.

The stimulator displaced the operculum with a sine-wave signal at a frequency of 2.0 kHz. This frequency was chosen to match the peak-response frequency of the TM in this species (Schoffelen et al. 2009b, chapter 4). The stimulus was switched on and off with a 100-ms sinusoidal ramp; the total duration of the stimulation was 2 s. Stimulus amplitudes ranged from -56dB to -20dB (all dB values are referenced to 1 µm, unless indicated otherwise), and were applied in an increasing sequence. For each stimulus level, we recorded a 3D movie of the response of the TM by taking images for eight stroboscopic phases and for 30 focal planes. For each of the 240 recorded images, the camera was active throughout the 2s-duration of a stimulus. The LED stroboscopically illuminated the specimen with a 10% duty cycle, except for the stimulus on and off ramp. After deactivation of the camera, the CCD’s data were read out and stored. The focal planes were spaced 1 µm apart and positioned progressively from the round-window side to the saccular side of the BP (fig. 5.1). The resulting 3D movie showed the response of the TM during a single period of the sinusoidal stimulation signal.

The total experimentation time per preparation was limited to three hours. For the first ear, that meant that all measurements were finished within four hours after killing the animal; for the second ear, it was within four hours after taking it out of the refrigerator, and within seven hours after the death of the animal. Visual inspection at the end of the experiment did not show degradation of the preparation. Also, no evidence of degradation of the TM response was found over such a period in earlier work with a similar method (Schoffelen et al. 2009b, chapter 4).

For data analysis, images were scaled to have a constant average gray value across the recorded phases and planes; the required correction factor was generally less than 10%. Next, the TM motion was analyzed using the ‘nD’ image-analysis package (Research Laboratory of Electronics, Massachusetts Institute of Technology, USA; Davis and Freeman 1998). Following the procedure described in Schoffelen et al. (2009b, chapter 4), we analyzed the motion in two-dimensional planes transecting the TM.

Three orthogonal directions x, y and z were defined relative to the microscope’s axis and the edge of the TM (see fig. 5.1). The z-direction was taken to be the viewing direction through the microscope; the y-direction was parallel to the edge of the TM in the image plane. The x-direction was perpendicular to both the y- and the z-direction.

To determine the TM displacement in the x- and z-directions, we took xz-cross sections of the 3D movie for a range of y-values containing the TM. Within each xz-plane a grid of 80x10 2D voxels was created; each voxel contained 17x3 pixels covering a 5.5 µm x 3 µm section.
Figure 5.2: Overview of amplitude and phase responses in three directions for various stimulus levels. The color code indicates the amplitudes obtained from the 2D cross sections (see Materials and methods); the data were smoothed by applying a 7x7 averaging window. The first and second columns indicate the displacement in the x- and y-directions, respectively. The third and fourths columns give both estimates of the z-displacement; the third column as calculated from the xz-cross sections, the fourth as calculated from the yz-cross sections. The stimulus frequency was 2.0 kHz. The gray vectors indicate the phase of the TM response at the location of their origin, relative to the stimulus signal; phase zero points to the right. A sketched indication of the lumen boundary, and of the location of the TM was drawn into the top left panel. Label: TM - tectorial membrane.
of the preparation. Using the nD package’s motion detection routines, the displacement of the features within each voxel was then determined for both of the perpendicular directions and for each of the eight phases. These displacements were averaged in the viewing direction (z). By fitting the displacements for the recorded phases to a sinusoid, the amplitude and phase of the corresponding motion was computed. These fitted values were then smoothed in the y-direction, which created the same resolution in the x- and y-directions. The same procedure was followed for yz-cross sections in order to analyze the response in the y- and z-directions. Due to the smaller image size in the y-direction, a 60x10 voxel grid was used. The combination of the results for both directions (xz and yz) produced one overview for the amplitude and phase for both the x- and the y-direction, and two for the z-direction.

For better quantitative analysis we used a region-of-interest (ROI) approach. Two or three 3D ROIs in the center of the TM were selected. These ROIs were selected to contain details of the TM’s structure; the presence of such details facilitates motion detection by the image-analysis software. The image analysis produced displacement estimations for each of the eight recorded phases and each direction (xyz). We averaged the displacements per direction and phase across the ROIs, and fitted them with a sinusoidal function. This gave a displacement amplitude and phase relative to the stimulus for each direction. Additionally, two or three ROIs in the limbic lumen boundary were analyzed in the same manner as the ROIs in the TM.

### 5.3 Results

In figure 5.2, color-coded plots are shown of the TM displacement amplitudes in one preparation for the various stimulus levels (rows), and for the three spatial directions (columns). The TM-response amplitude increased with growing stimulus amplitude; both the peak amplitude and the displaced area increased. For each direction, the peak response occurred in the lower part of the TM (if it’s oriented with the TM’s edge at the top, fig. 5.1d,e), near the hair bundles. These responses were lower for the directions in the image plane (xy) than they were along the line of sight (z). The TM’s edge remained almost still, just like the lumen boundary.

Baseline measurements, with zero stimulus amplitude, showed a pattern corresponding to that of the measurements at the -56 dB stimulation level. The noise was noticeably higher in the viewing direction (z) of the microscope than in the in-plane directions (xy). This is presumably due to the lower resolution of the data-set and the lower image sharpness along the line of sight.

The phase of the TM motion is indicated by the gray arrows in figure 5.2. The phase relative to the input signal is approximately constant in the displaced area of the membrane for all three directions. At low TM-response amplitudes (<-30 dB) the picture is, as expected, more noisy than at higher amplitudes. The phase of the displaced area in the x-direction is approximately the same as in the y-direction, while it has a phase difference of approximately 180° with the z-direction.

Using the ROI-analysis, we determined the displacements more quantitatively in the three spatial directions. Figure 5.3 gives an overview of the displacements of both the TM and the lumen-boundary amplitudes for four preparations at the various stimulation levels.

For stimulus levels below -30 dB, the TM response grew approximately linearly (1dB/dB) in all four preparations in the x-direction, and for three out of four preparations in the z-
Figure 5.3: I/O curves for four preparations. The three spatial components are indicated by different symbols: the circles indicate the amplitude in the x-direction, the squares in the y-direction, and the crosses in the z-direction. The dashed lines indicate the response of the lumen boundary. The responses increased with increasing input levels.

Above -30 dB stimulus levels, these responses tended to saturate in two preparations, and even decrease in one. In three preparations, the response in the y-direction was similar to that in the z-direction. In one case (prep. D), the TM response did not exceed the lumen boundary's. The deviation in the y-direction response was presumably caused by the variation in the viewing angle onto the TM between preparations (see Discussion, section 5.4). Typically, the TM responses were higher in the viewing direction (z) and in the x-direction than they were in the y-direction. In the z-direction, the maximum responses ranged from -5 dB to -30 dB; in the x-direction from -20 dB to -32 dB; and in the y-direction from -23 dB to -40 dB.

In addition to the TM amplitudes, figure 5.3 shows the amplitudes of the ROIs chosen in the lumen boundary. Generally, the amplitude of the lumen boundary was lower than the TM response. In the z-direction the response amplitude was relatively constant, while it tended to increase slightly with increasing stimulus amplitude in the x- and y-directions.

The noise floor was determined for the combination of the measurement method and analysis technique from measurement data at zero stimulation amplitude. The noise floor was, on average, -61 dB (± 6 dB, standard deviation; including both TM and lumen ROIs) for the in-image-plane directions (xy), and -49 dB (± 7 dB) in the z-direction. The difference between these noise-floor values is presumably due to the difference in resolution.

For all three directions, the phase difference was approximately constant across stimulus levels (omitting the data point at -56 dB). In three preparations (B, C, D; fig. 5.4), the x- and y-components of the TM motion had the same phase, and they were about 180° out of phase with the z-component. In one case (prep. A), the y- and z-motion had the same phase, while the x-motion was about 180° out of phase. The relative phases are explicitly shown in figure 5.5. Excluding the measurements at the -56-dB stimulus level, the average phase difference between the x- and z-components was 155°±32° (sd); between the x- and y-directions it was...
Figure 5.4: Phases of the response relative to the stimulus. The three spatial components are indicated by different symbols; the circles indicate the amplitude in the x-direction, the squares the y-direction, and the crosses the z-direction. Phase differences between various components were either approximately 0° or approximately 180°.

5.5. Excluding the measurements at the -56-dB stimulus level, the average phase difference between the x- and z-components was 155°±32° (sd); between the x- and y-directions it was 14°±24°. The amplitude levels and these phase differences are consistent with a mode of motion along the sensory epithelium of the BP (see Discussion, and fig. 5.8).

Figure 5.6 again shows the TM’s amplitude response to operculum displacement. Here, the displacement amplitudes for the TM and the lumen boundary were averaged across the preparations. For the y-direction average, the data from preparation D were excluded from the calculation (see Discussion). The I/O curves in figure 5.6 showed the same overall trend as the separate panels in figure 5.3: At stimulus levels below -30 dB the TM amplitudes depended linearly the input amplitudes. In the linear range, the TM response exceeded the stimulus amplitude by about 3 dB in the x- and z-directions. In the y-direction, the TM response was approximately the same as the input amplitude. At stimulus levels above -30 dB, the responses tended to saturate.

The averaged displacement amplitudes of the lumen boundary (fig. 5.6) were relatively constant in the x- and z-directions. In the y-direction, the lumen boundary’s I/O curve paralleled the TM’s at a level about 8 dB lower.

5.4 Discussion

The data presented above showed that the response in all three spatial directions increased with increasing stimulus amplitudes. The maximum displacements occurred near the hair bundles, and the response phase is approximately constant across the TM. The smallest amplitudes were observed near the edge of the TM opposite the hair cells. These findings were consistent with earlier findings (Schoffelen et al. 2009b, chapter 4): the TM’s motion is
Figure 5.5: Phase differences between orthogonal components of the response. Excluding the lowest stimulus level, the phase difference between the x- and y-directions was close to 0° for three preparations, and about 180° for one. The phase difference between the x- and z-directions was close to 180° for all four preparations.

Figure 5.6: Averaged I/O curves for the three spatial directions. The solid lines indicate the TM response. The dashed black lines indicate the response of the lumen boundary. The error bars indicate the standard deviation of the response at that stimulus level. The gray dashed lines correspond a 3 dB amplification of the stimulus signal. The y-direction data for preparation D were not included in this graph, as explained in the Discussion section.
similar to that of a two-dimensional pendulum, hinged at the TM’s edge.

The amplitudes of motion depended linearly on the operculum displacement up to a stimulus amplitude of about -30 dB. The TM responses saturated above this level. The neural responses in *R. pipiens* saturate at approximately 80 dB SPL (instantaneous rates; Ronken 1990). In order determine whether neural saturation is related to the mechanical saturation of the TM, we needed to relate our stimulus levels at the operculum to the sound-pressure levels used by Ronken. Two approaches were used to relate the sound-pressure level at the tympanic membrane, to our displacement amplitudes at the operculum.

The first approach is based on transfer from sound-pressure at the tympanic membrane to motion at the operculum. In the bullfrog, a stimulus of 90 dB SPL at the tympanum corresponds to a maximum operculum velocity of -23 dB (re. 1 mm/s) within the bullfrog’s BP frequency range (Mason and Narins 2002b). Assuming the same transfer ratio between the sound-pressure level and the operculum velocity showed that our stimuli ranged from the equivalent of 78 dB SPL to 115 dB SPL.

Alternatively, we could estimate the sound-pressure level corresponding to our stimuli by considering the hair-bundle deflection at the neural threshold. The detection threshold for hair cells is approximately a 1 nm deflection (-60 dB) of the hair bundle (Hudspeth 1997). In the leopard frog’s BP, the auditory threshold is achieved by a 60 dB SPL stimulus (Ronken 1990). With the TM responses in the x- and z-directions 3 dB above the stimulus (dashed gray lines, fig. 5.6), our stimuli corresponded to 66 to 103 dB SPL by this second approach. By averaging the results of both approaches, we estimated that the range of our stimuli to corresponded to sound-pressure levels between 72 and 109 dB SPL.

In figure 5.7 the averaged I/O curves (fig. 5.6) are plotted against the estimated dB-SPL scale. Saturation of the TM response occurred around 100 dB SPL, while the instantaneous neural spike rate saturates at 80 dB SPL (gray lines; based on Ronken 1990). So, although the tuning of the BP is based on the mechanical response of the TM (Schoffelen et al. 2009b, chapter 4), the upper limit of the neural response must be determined by the hair-cell and auditory-nerve physiology.

The averaged response in the y-direction was non-linear, due to the measurements from one preparation. In this preparation, the y-direction TM response did not exceed the lumen boundary response (fig. 5.3, prep. D). This may have been the result of a coincidental perfect alignment of the xz-planes with the motion of the TM in this specific measurement. For this reason, we excluded the data from this preparation from the averaged I/O curve for the y-component in figure 5.6. In the other preparations, the y-direction response was similar to that in the x- and z-directions, indicating that the TM had a component of motion perpendicular to the xz-plane.

The magnitude of the composite TM motion exhibited a similar behavior as the individual components (black dashed line in fig. 5.7): a linear response up to between 95 and 100 dB SPL, and saturation for higher stimulus levels. In the linear range, the gain between the operculum and the TM displacements was approximately 8 dB. This gain was slightly lower than that reported for gain at the best frequency of the TM response (~ 15 dB, Schoffelen et al. 2009b, chapter 4). This difference is presumably due to the fact that our measurements were obtained from ROIs in the center of the membrane, which has a lower amplitude than the area near the hair cells that was used by Schoffelen et al.. Also, the gain they determined was at the known best frequency of the TM, while our measurements may have been performed close to, but not quite at, the best frequency.
Figure 5.7: TM I/O curves compared to nerve fiber I/O curves (Ronken 1990). The gray lines indicate the auditory nerve’s spike rate; solid the instantaneous rate, and dashed the sustained rate. The spike rates are relative to the left axis. The averaged TM I/O curves are displayed for three directions. The circles represent the x-direction, the squares the y-direction and the crosses the z-direction. The dashed black line indicates the total TM displacement assuming that the phase difference between spatial components was either 0° or 180°. The TM displacements are relative to the right axis, in dB re. 1 µm. 


In figure 5.8, an xz cross section through the BP of the northern leopard frog is displayed (light microscopy, previously unpublished data; chapter 3). The viewing direction is drawn (z) assuming that we look onto the contact membrane perpendicularly, with the x-direction perpendicular to it. The double-headed arrow indicates the direction of motion within this plane, in case the magnitudes of the x- and z- components are the same (fig. 5.6) and their phase-difference is 180° (fig. 5.5). In the image this direction is then approximately parallel to the hair-cell excitatory direction. Therefore, it is likely that the TM’s mode of motion was along the epithelium, parallel to the hair-bundle orientation.

As indicated in the Material and Methods section, the data presented in this paper were obtained from isolated post-mortem preparations of the frog inner ear. The time frame of the experiment was after distortion-product otoacoustic emissions from the organ disappear (approx. 30 min post mortem, Van Dijk et al. 2003), which excludes active hair-cell involvement on the responses. Inspection after the measurements showed no visible degradation of the BP’s structure. However, the ringer solution in which the preparation was submerged contained increasingly more debris over the duration of the experiment, presumably due to the vibration of the stimulator and the operculum. We limited the experimentation time to three hours for each preparation, and conducted no measurements longer than seven hours after killing the animal. In earlier work (Schoffelen et al. 2009b, chapter 4), no degradation of the response was witnessed over similar measurement durations, and comparable stimulus
Figure 5.8: Illustration of the motion of the TM, superimposed on a light-microscopy image of an xz-cross section of the BP. The z-direction indicates the viewing direction through the microscope’s objective. The double-headed arrow indicates the calculated xz-motion of the tectorial membrane from our data. The 45° angle resulted from the amplitudes being the same in both directions (fig. 5.6) and a 180° phase difference between them. The photo was positioned under the assumption that the microscope’s viewing direction was perpendicular to CM. The hair-cell polarization is then approximately parallel to the measured TM motion. Labels: CM - contact membrane, HED - hair-cell excitatory direction.

levels. This suggests that the structural properties of the isolated inner ear maintained a stable, normal state throughout the experiment, while any active hair-bundle involvement had probably ceased before the start of the experiment.

In conclusion, our data showed that the tectorial membrane’s motion in the basilar papilla of the northern leopard frog is most likely along the basilar papilla’s epithelium surface. It aligns with the hair bundle orientation, and thus deflects the hair bundles in the most effective manner. The tectorial membrane’s response amplitude increased linearly with the displacement of the operculum (oval window) for stimulation levels up to an equivalent of approximately 100 dB SPL. Since the neural response saturates at lower levels (approximately 80 dB SPL, Ronken 1990), the mechanical response of the tectorial membrane does not determine the maximum nerve-fiber response.
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