

Sound and vibration sensitivity of VIIIth nerve fibers in the frogs *Leptodactylus albilabris* and *Rana pipiens pipiens*

J. Christensen-Dalsgaard*, P.M. Narins

Department of Biology, UCLA, 405 Hilgard Avenue, Los Angeles, CA 90024, USA

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Abstract. 1. Responses of 73 fibers to dorso-ventral vibration were recorded in the saccular and utricular branchlets of *Rana pipiens pipiens* using a ventral approach. The saccular branchlet contained nearly exclusively vibration-sensitive fibers (33 out of 36) with best frequencies (BFs) between 10 and 70 Hz, whereas none of the 37 fibers encountered in the utricular branchlet responded to dorso-ventral vibrations.

2. Using a dorsal approach we recorded from the VIIIth nerve near its entry in the brainstem and analyzed responses to both sound and vibration stimuli for 65 fibers in *R. pipiens pipiens* and 25 fibers in *Leptodactylus albilabris*. The fibers were classified as amphibian papilla (AP), basilar papilla (BP), saccular or vestibular fibers based on their location in the nerve. Only AP and saccular fibers responded to vibrations. The AP-fibers responded to vibrations from 0.01 cm/s² and to sound from 40 dB SPL by increasing their spike rate. Best frequencies (BFs) ranged from 60 to 900 Hz, and only fibers with BFs below 500 Hz responded to vibrations. The fibers had identical BF's for sound and vibration. The saccular fibers had BFs ranging from 10 to 80 Hz with 22 fibers having BFs at 40–50 Hz. The fibers responded to sound from 70 dB SPL and to vibrations from 0.01 cm/s².

3. No differences in sensitivity, tuning or phase-locking were found between the two species, except that most BP-fibers in *R. pipiens pipiens* had BFs from 1.2 to 1.4 kHz, whereas those in *L. albilabris* had BFs from 2.0 to 2.2 kHz (matching the energy peak of *L. albilabris*' mating call).

4. The finding that the low-frequency amphibian papilla fibers are extremely sensitive to vibrations raises questions regarding their function in the behaving animal. They may be substrate vibration receptors, respond to sound-induced vibrations or bone-conducted sound.

Key words: Vibration sensitivity – Hearing – Eighth nerve – Frog – *Rana pipiens pipiens* – *Leptodactylus albilabris*

Introduction

The inner ear of the frog possesses a remarkable sensitivity to substrate-borne vibrations. In the 3 species studied to date significant changes in spike-rate of inner ear afferents have been observed at acceleration amplitudes as low as 0.01 cm/s² (Koyama et al. 1982; Narins and Lewis 1984; Christensen-Dalsgaard and Jørgensen 1988; review in Narins 1990), placing the frogs among the most vibration-sensitive animals studied.

The most sensitive frog species studied so far, the Puerto Rican white-lipped frog *Leptodactylus albilabris* is also reported to use vibration signals in intraspecific communication. Lewis and Narins (1985) observed that calling males emit a substrate-borne “thump” when their vocal sac strikes the substrate and showed that artificial thumps altered male calling behavior. Narins (1990) suggested that vibrational communication in this frog utilizes a relatively noise-free channel compared to airborne sound, where the background noise level typically is 80–90 dB SPL. For the other frog species studied, however, no behavioral use of the exquisite vibration sensitivity has been reported.

Recordings from the saccular nerve in *Rana temporaria* showed that nearly all afferents in this branchlet are vibration-sensitive with best frequencies (BFs) ranging from 10 to 80 Hz (Christensen-Dalsgaard and Jørgensen 1988). Jørgensen and Christensen-Dalsgaard (1989, 1991) recorded from the anterior and posterior branches of the VIIIth nerve and found vibration-sensitive fibers in both branches. The anterior branch innervates the sacculus, utriculus and the anterior and horizontal semicircular canals; the posterior branch innervates the amphibian and basilar papillae, lagena and the posterior semicircular canal. In the anterior branch

* Present address: Institute of Biology, Odense University, Campusvej 55, DK-5230 Odense M, Denmark

Abbreviations: SPL, sound pressure level; BF, best frequency; AP, amphibian papilla; BP, basilar papilla

Correspondence to: J. Christensen-Dalsgaard

BFs ranged from 10 to 100 Hz with most fibers having BFs at or below 40 Hz; in the posterior branch BFs were relatively uniformly distributed in the range from 10 to 300 Hz. Since they found no vibration-sensitive fibers in the lagenar nerve they concluded that the vibration-sensitive fibers in the posterior branch originate in the amphibian papilla. They also suggested that some of the utricular afferents may be vibration-sensitive. Recently, Yu et al. (1991) studied responses of VIIIth nerve afferents to sound and vibration in *Rana catesbeiana*. Using a dorsal approach to the VIIIth nerve (thus allowing the frog to sit in a normal posture) they measured tuning curves for sound and vibration in AP and saccular fibers using reverse correlation. They showed that low-frequency amphibian papilla afferents also in this species are quite sensitive to vibrations and, conversely, that saccular afferents also respond to sound as reported earlier by Moffat and Capranica for *Bufo americanus* (1976).

It has been shown that low-frequency auditory fibers in the northern leopard frog *R. pipiens pipiens* can be stimulated with sound via a non-tympanic route which is probably more efficient than the tympanic route at frequencies below 300 Hz in a free sound field (Wilczynski et al. 1987). The mechanism of this "extratympanic" sensitivity is presently unknown, but the considerable sensitivity of the low-frequency AP fibers to whole-body vibrations might be expected to contribute to the extratympanic sensitivity, as suggested by Jørgensen and Christensen-Dalsgaard (1991).

In the present study we wanted to compare directly the sensitivity to sound and vibrations of single VIIIth nerve afferents in two frog species, the northern leopard frog *Rana pipiens pipiens* and the white-lipped frog *Leptodactylus albilabris*. *R. p. pipiens* is not known to communicate with vibrational signals and by comparison it might be possible to identify any special adaptations for vibrational communication in the VIIIth nerve of *L. albilabris*. Furthermore, by comparing sound and vibration responses we hoped to assess the importance of tympanic and non-tympanic inputs to the ear of the two species.

Methods

We used adult northern leopard frogs (*Rana pipiens pipiens*) obtained from a commercial dealer and Puerto Rican white-lipped frogs (*Leptodactylus albilabris*) caught in the Luquillo Mountains in the Caribbean National forest in eastern Puerto Rico.

The animals were anesthetized using intramuscular injections of Nembutal [sodium pentobarbital (60–100 µg/g body weight)]. After disappearance of reflexes, one of two dissection procedures was used. The first procedure utilized a ventral approach through the roof of the mouth. A small hole was made in the otic capsule, exposing the saccular branchlet and in some cases also the utricular branchlet (see Feng et al. 1975).

In the second procedure the VIIIth nerve was exposed dorsally. A midline incision was made in the skin on the head at the level of the tympanum. A skin flap was reflected laterally to expose the posterior part of the frontoparietal bone. The muscles longissimus dorsi and rhomboideus capitus were detached from the frontoparietal bone and retracted to expose the exoccipital bone. We

used a dental drill to thin the exoccipital bone and removed the last part of the bone with scalpel and fine hooks, thus exposing the dura and the choroid plexus. The choroid plexus and the vertebral artery were then retracted medially with a small hook fixed in the frog's skin. Thus, the VIIIth nerve was exposed with minimal bleeding at its entry in the brainstem. Supplemental doses of Nembutal were administered as needed during the experiments. In addition, we injected the frogs with d-tubocurarine chloride (2–3 µg/g body weight) to ensure immobilization during the experiments. The frogs normally recovered well from the surgery.

For the recordings the frog was placed either on its back (ventral approach) or in a natural posture (dorsal approach) on a platform mounted on a vibration exciter (Brüel and Kjaer 4809). The vibration exciter was placed in a small sound-isolated Faraday cage set on top of a five-stage vibration isolation table. This consisted of a commercial vibration isolation table (Backer-Loring Micro-g) supporting a stack of alternating layers of heavy steel plates and 10 cm thick foam rubber mattresses. This arrangement effectively attenuated vibrations in the setup to below 0.001 cm/s² (20 dB below the lowest level used) within the frequency range used (10–2550 Hz).

A search stimulus was presented during the VIIIth nerve penetration. This stimulus was a frequency sweep (10–2500 Hz, 2 octaves/s) presenting sound and vibrations simultaneously at levels of 75 dB SPL and 0.25 cm/s², respectively. When a fiber was encountered, its best frequency (BF) and threshold to sound and vibration was determined. After that, a series of sound and vibration stimuli was run, first at the BF and later (time permitting) at other frequencies.

Vibration and sound stimuli were continuous sinusoids (10–2550 Hz) presented for 15 or 30 s. The sinusoids were produced by a low-distortion tone generator (Wavetek 186). Signal levels could be attenuated in 1.5 dB increments by a computer-controlled digital attenuator. The sinusoidal signal was then split into a sound and a vibration channel, passed to two manual attenuators (HP 350D), to the two channels of a Power Envelope stereo amplifier (NAD 2100) and finally to the vibration exciter and headphone. This configuration facilitated switching between the two stimulus modalities. Both the tone generator and the digital attenuator were controlled by an 80386 PC using custom-made hard- and software. Using the digital attenuator the vibration stimuli were equalized, so accelerations were constant within 1.5 dB in the frequency range used. The sound stimuli had a maximal variation of 6 dB with frequency; in the data, stimulus levels were corrected for this variation.

Peak acceleration amplitudes for the vibrational stimuli ranged from 0.01 to 10 cm/s². For the sound stimuli r.m.s. sound pressure levels ranged from 40 to 110 dB SPL. The vibration stimulus was monitored continuously with an accelerometer (B&K 4370) placed near the frog's head. Closed-field sound stimulation was delivered by a headphone (Beyer DT 48) in a closed brass coupler that was sealed around the frog's eardrum with vaseline. Sound levels inside the coupler were measured with a calibrated half-inch microphone (B&K 4134) with a probe extension.

The vibrations delivered by the coupler to the frog's head were measured using a laser doppler vibrometer (Polytec OFV501) at 50, 100 and 200 Hz at 115 dB SPL. The maximal acceleration measured was 2.4 cm/s² at 200 Hz. At 50 and 100 Hz peak accelerations were 0.18 cm/s² and 0.72 cm/s², respectively. Thus, only at sound levels above approx. 90 dB were the vibrations above threshold for the most sensitive fibers. At these levels, we always controlled that the sound induced vibrations at threshold were at least 20 dB below the vibration thresholds.

The sound emission from the vibration exciter platform was measured earlier (Christensen-Dalsgaard and Jørgensen 1989) and it was found that sound levels at the level of the frog's tympanum ranged from 52 to 56 dB SPL at an acceleration amplitude of 13 cm/s² for frequencies in the 100–600 Hz range. Consequently, the sound levels emitted in these experiments ranged from –4 dB SPL at 0.01 cm/s² to 53 dB SPL at 10 cm/s² and would only stimulate very sound sensitive fibers at the highest vibration levels (lowest thresholds to closed field sound was 40 dB SPL).

Single unit data were recorded using conventional glass microelectrodes (20–40 MΩ). Action potentials were conditioned by a unity-gain microelectrode preamplifier (Winston Electronics 1090) and amplified 1000 times (OpAmp Labs, model 423). The responses were filtered and passed to a comparator controlled by the 386 PC. When triggered by an action potential, the comparator latched a 24-bit timer (counting microseconds) and restarted the timer. As a result, the time intervals between spikes were measured with one microsecond resolution. The latched value was then read by the computer and for each spike the 24-bit value was stored on disk. During data collection the computer generated dotplots and following a stimulus presentation, interval histograms were assembled and displayed. All computer interfaces and software for the data acquisition were custom-made.

To facilitate comparison of sound and vibration levels, in both cases the units of measurement were converted to velocities. The purpose of this conversion was to compare rate-level curves with the same dB reference level. For the platform vibrations velocities v_p were calculated from the measured accelerations a_p by dividing with $(2\pi f)$ where f is the stimulus frequency.

The particle velocity v_c of sound was calculated from the sound pressure p in the coupler as

$$v_c = \frac{p}{\rho_{air} \cdot c_{air}}$$

where ρ_{air} is the density of air and c_{air} is the speed of sound in air. The particle velocity calculated in this way is *not* the actual particle velocity in the coupler, but rather the free field particle velocity corresponding to the measured sound pressure.

Finally, the eardrum vibrations at the sound pressures used were calculated. The calculations were based on the results of Vlaming et al. (1984). They measured eardrum vibrations in *Rana temporaria* using laser vibrometry and closed-field sound stimulation and found a low-pass characteristic for tympanic displacement with a constant displacement of approximately 50 nm at 80 dB SPL in the frequency range of 100 to 1000 Hz. Here, we assumed constant displacement down to 40 Hz.

Results

We recorded responses of 73 fibers in the anterior branch (saccular and utricular branchlets) of the VIIIth nerve of

12 *R. pipiens pipiens* using a ventral approach. Furthermore, using a dorsal approach we recorded responses of 65 and 25 cells, respectively, in the VIIIth nerve of 8 *R. p. pipiens* and 6 *L. albilabris*.

Saccular and utricular branchlets

In *R. p. pipiens*, we recorded from 37 fibers in the anterior branch lateral to its merge with the saccular branchlet, but we did not find any vibration-sensitive fibers. This part of the anterior branch innervates the utricle and two semicircular canals. In contrast, 33 of 36 fibers encountered in the saccular branchlet responded to low-frequency vibrations; the remaining 3 fibers showed a very regular spontaneous activity and may have been efferents.

At low stimulus levels, the saccular fibers did not increase their average spike rate above the spontaneous activity, but the probability of firing was modulated by the stimulus sinusoid so the preferred intervals between discharges are integer multiples of the cycle time. At higher stimulus levels the average spike rate was increased up to a maximum of 1 spike/cycle and nearly all discharges occur at integer multiples of the cycle time. Figure 1 shows the responses of a saccular fiber stimulated at an acceleration level of 0.25 cm/s² at 5 different frequencies. The threshold is defined as the stimulus level producing a just noticeable increase in spike rate above the spontaneous level. For this fiber, the vibration threshold was approx. 0.05 cm/s² at its BF (50 Hz). The saccular fibers generally phase-lock to frequencies up to at least 200 Hz. However, for the fiber response shown in Fig. 1, the interval histograms show that as frequencies are increased the fibers tend not to discharge on every stimulus cycle, but rather skip one or more cycles, producing multiple peaks in the interval histogram. If the interspike intervals are used by the CNS for frequency

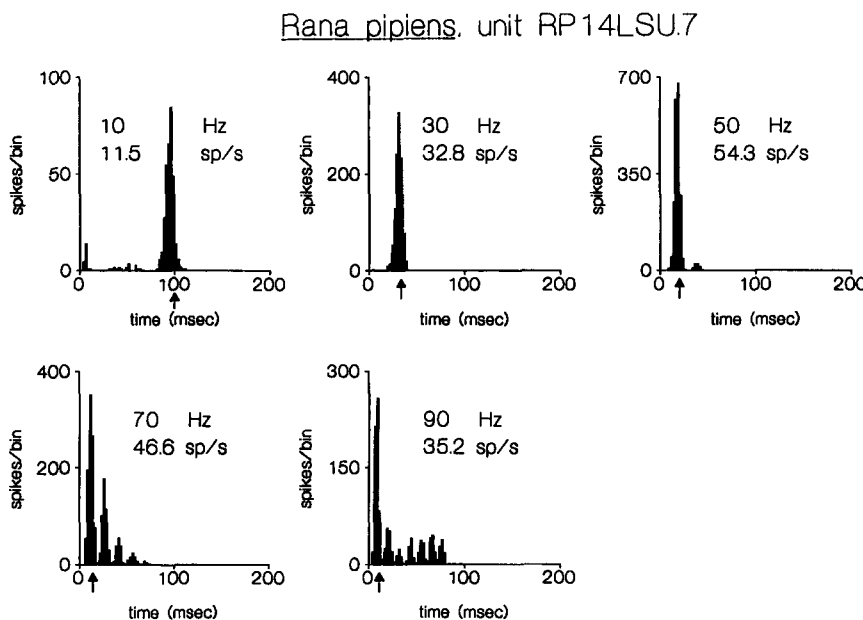


Fig. 1. Interval histograms of a saccular fiber's response to dorsoventral vibrations at 5 different frequencies at 0.25 cm/s². In each histogram, the upper number is the frequency (in Hertz) and the lower number is the mean spike rate (in spikes/s). Cycle interval is marked by an arrow

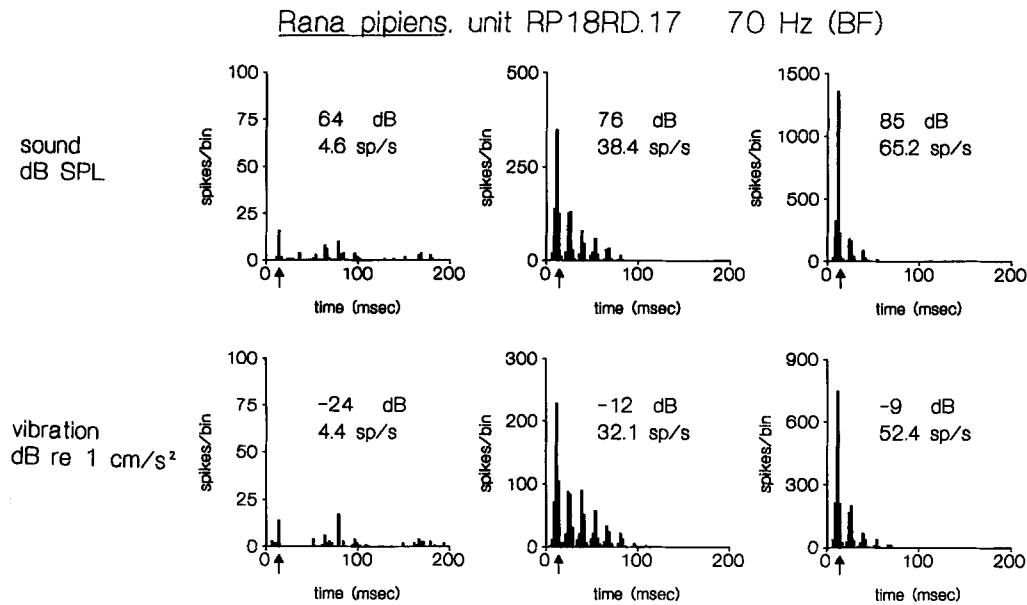


Fig. 2. Interval histograms of the response of an AP-fiber from *R. p. pipiens* to sound (*top row*) and vibration (*bottom row*) stimulation at its BF (70 Hz) at 3 sound and acceleration levels. In each histogram, the upper number is the sound or vibration level in dB SPL (*top row*) or dB re 1 cm/s² (*bottom row*) and the lower number is the mean spike rate (in spikes/s). Cycle interval is marked by an arrow. Note the different ordinates

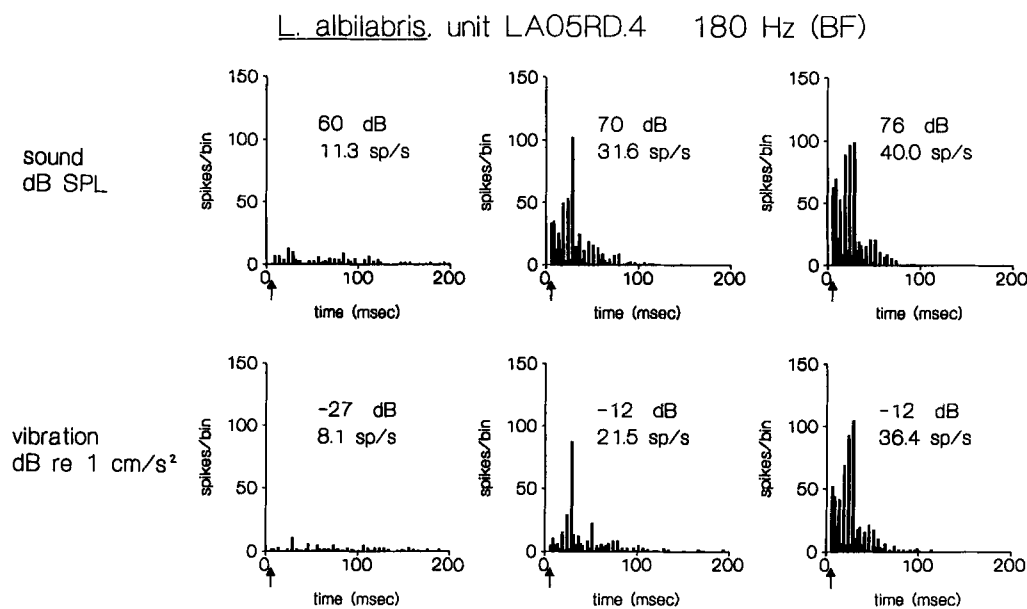


Fig. 3. Interval histograms of the response of an AP-fiber from *L. albilabris* to sound (*top row*) and vibration (*bottom row*) stimulation at its BF (180 Hz) at 3 sound and acceleration levels. In each histogram, the upper number is the sound or vibration level in dB SPL (*top row*) or dB re 1 cm/s² (*bottom row*). Cycle interval is marked by an arrow

analysis, this effect would create ambiguity by introducing frequencies lower than the stimulus frequency. The fiber in Fig. 1 exhibited a mean spontaneous rate of approximately 18 spikes/s; thus, the firing rate is reduced at the lowest frequency tested (to 11.5 spikes/s).

Generally, the saccular fibers had best frequencies (BFs) ranging from 10 to 70 Hz and spike-rate vibration thresholds ranging from 0.01 to 1.0 cm/s².

VIIIth nerve

Using a dorsal approach, 4 groups of fibers were found in the VIIIth nerve of *R. p. pipiens* and *L. albilabris*. The first group responded to low-frequency airborne sound and dorso-ventral vibrations with BFs from 70 to

900 Hz. Based on their dorsal location in the nerve, these fibers were classified as amphibian papilla-fibers (AP-fibers). The second group was found more ventrally and responded only to high-frequency sound with BFs typically in the range of 1.2 to 1.5 kHz (*R. p. pipiens*) and 2.0 to 2.3 kHz (*L. albilabris*). These cells were classified as basilar papilla-fibers (BP-fibers). The third group, found still more ventrally, responded to dorso-ventral vibrations and airborne sound with BFs in the range of 30–80 Hz. Based on comparisons with our recordings from fibers in the saccular branchlet of *R. pipiens* (see above), these fibers were classified as saccular. Finally, a fourth group of fibers ventralmost in the nerve did not respond to sound or vibrations and were tentatively classified as vestibular.

The spontaneous rate of all VIIIth nerve fibers ranged

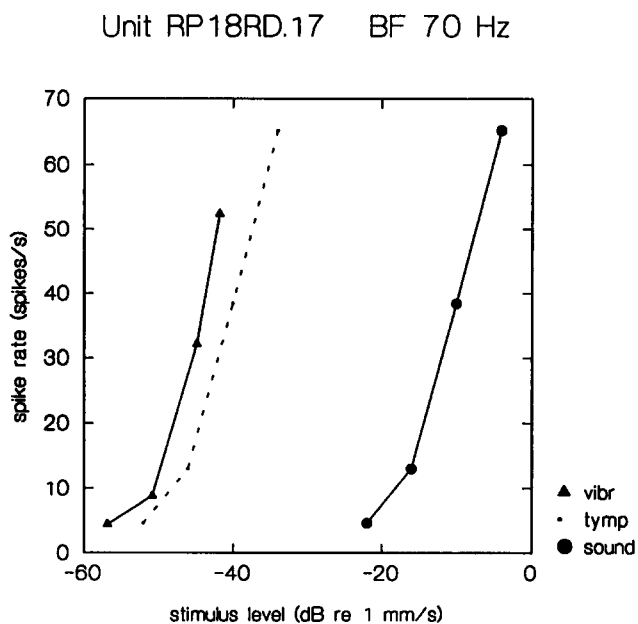


Fig. 4. Rate-level curves of an AP-fiber from *R. p. pipiens* stimulated with sound (circles) and with dorso-ventral vibration (triangles) at its BF (70 Hz). All levels are dB re 1 mm/s. Eardrum vibrations at the sound levels used are calculated (dashed curve). See Methods for further details

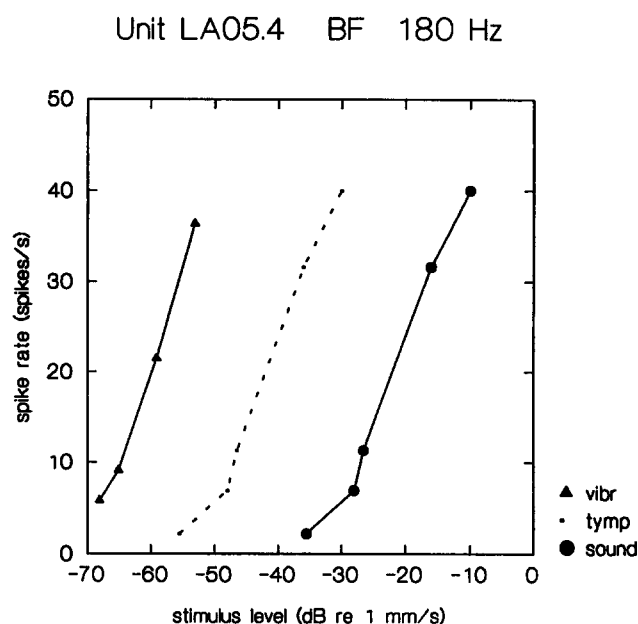


Fig. 5. Rate-level curves of an AP-fiber from *L. albilabris*. Other details same as Fig. 4

from 0 to 30.8 spikes/s. There was no clear difference between spontaneous rates of the saccular and the AP fibers.

Amphibian papilla fibers

36 fibers from the two frog species were classified as AP fibers. In both species, the fibers were organized tonotopically with the lowest frequencies dorsally in the VIIIth nerve. Just after penetration of the neural sheath of the VIIIth nerve, a population of fibers with low BFs (typically just below 100 Hz) and very sharp phase-locking was found. These cells were, however, quite difficult to hold, perhaps because the axons have small diameters (and, consequently, few of our recordings come from this population). In contrast, the following layer of fibers with BFs ranging from approximately 120 to 300 Hz were notably easier to hold and thus, most of the analyzed responses come from this population, although we found fibers with BFs which spanned the range from 70 to 900 Hz.

Figure 2 shows an example of the response of a low-frequency AP-fiber found dorsally in the VIIIth nerve of *R. p. pipiens*. The fiber had the same BF for sound and vibration. The figures are interval histograms of the responses to sound and vibration at the BF (70 Hz). The fiber had a threshold to airborne sound of approx. 64 dB SPL and responded to vibrations from levels of approx. 0.06 cm/s². The responses to sound and vibration are very similar. Again, the preferred intervals of discharges are integer multiples of the cycle time (shown by arrows), indicating phase-locked discharges.

Figure 3 shows the response of an AP-fiber in *L. albilabris*. The BF is 180 Hz. The threshold is approximately 60 dB SPL for sound and 0.07 cm/s² for vibration. This fiber is representative of the AP-fibers with BFs from 120 to 300 Hz, that is, extremely sensitive to both sound and vibrations with phase-locked discharges. The interval histograms are more complicated than those of fibers with lower BFs, because these fibers rarely discharge at every stimulus cycle, even at high stimulus levels.

The lowest thresholds of the AP-fibers were at approx. 40 dB SPL for sound and 0.01 cm/s² for vibrations for both *R. p. pipiens* and *L. albilabris*. We saw no differences between the two frog species in terms of sound/vibration sensitivity, phase-locking or tuning of the fibers (except in the BFs of the BP fibers as stated above). We could not elicit a response in AP-fibers with BFs above 500 Hz with vibrations at the levels used (maximum 10 cm/s²). Similarly, the BP-cells did not respond to vibration even at the BF (for sound) at the vibration levels used.

To compare quantitatively the responses of the fibers to sound and vibrations, we plotted the rate-level curves for the same fiber stimulated with sound and vibration. An example is shown in Fig. 4. The fiber was an AP fiber from *R. p. pipiens* with a BF of 70 Hz. As seen in the figure, sound and vibration stimulation produce nearly identical sigmoidal rate-level curves. When both sound and vibration levels are calculated as velocities to facilitate comparison (see Methods), the offset between the two rate-level curves (hereafter called the S-V offset) is approx. 35 dB for this fiber. For this fiber, the rate-level curves for the measured platform vibration and the calculated eardrum vibration are nearly coincident.

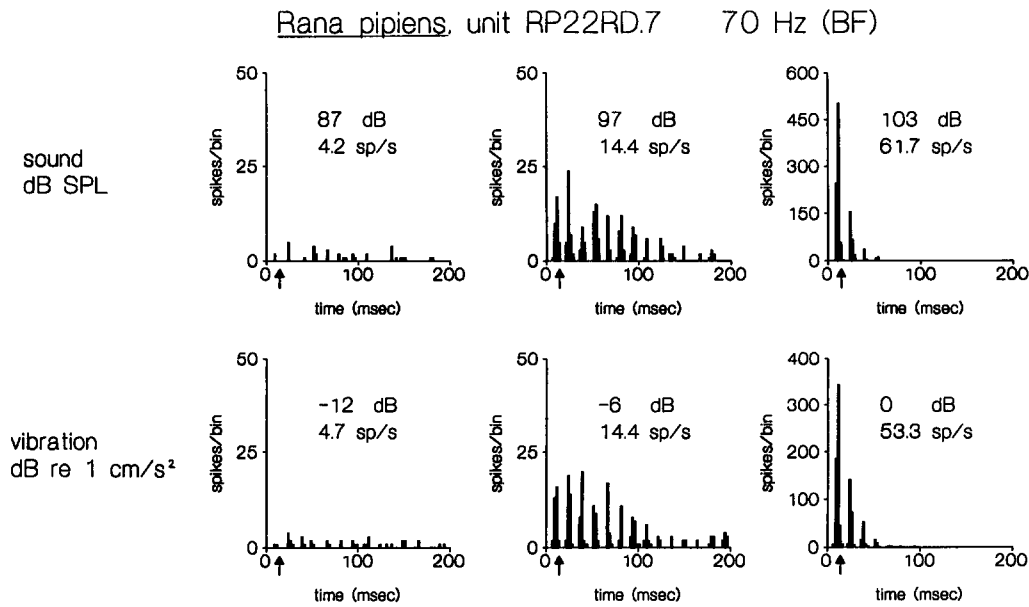


Fig. 6. Interval histograms of the response of a saccular fiber from *R. p. pipiens* stimulated with sound (*top row*) and vibrations (*bottom row*) at its BF (70 Hz) at 3 sound and acceleration levels. In each histogram, the upper number is the sound or vibration level in dB SPL (*top row*) or dB re 1 cm/s² (*bottom row*). The lower number is the mean spike rate (in spikes/s). Cycle interval is marked by an *arrow*. Note the different ordinates

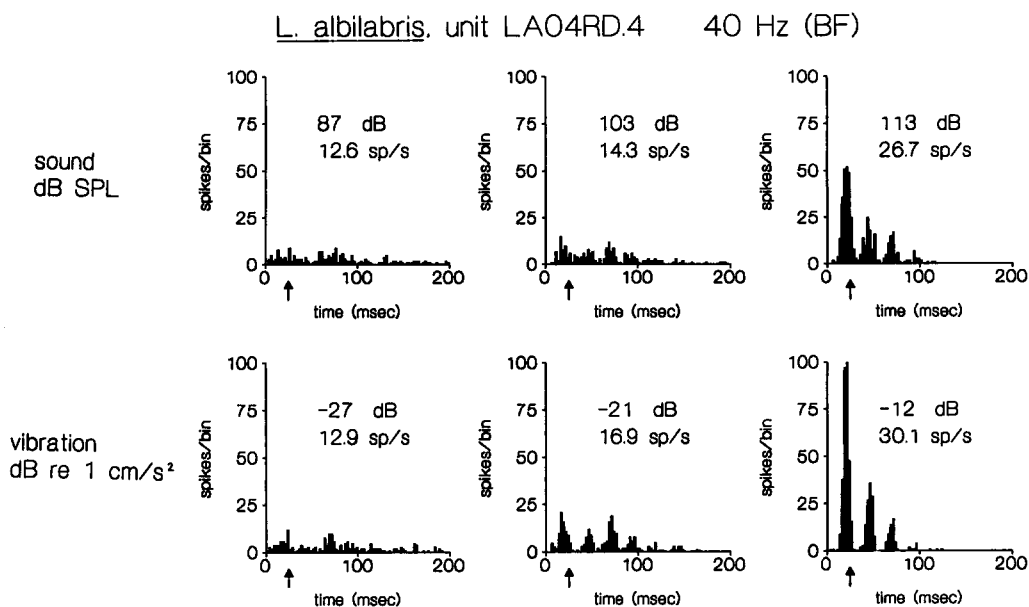


Fig. 7. Interval histograms of the response of a saccular fiber from *L. albilabris* with a BF of 40 Hz. Other details same as Fig. 6

Figure 5 shows rate-level curves from an AP-fiber with higher BF (180 Hz) in *L. albilabris*. Again, sound and vibration produces nearly identical sigmoidal rate-level curves. Here, the S-V offset is approximately 38 dB; the offset between the platform vibration and the eardrum vibration rate-level curves is approximately 15 dB. For the other AP-fibers in both *R. p. pipiens* and *L. albilabris* the S-V offset between vibration and sound rate-level curves ranges from 10 to 50 dB.

Saccular fibers

39 fibers from the two frog species were classified as saccular. The saccular fibers were found below the BP

fibers. They all had BFs ranging from 10 to 80 Hz and 22 fibers in both *R. p. pipiens* and *L. albilabris* had BFs between 40–50 Hz. Again, for the saccular fibers we found no differences in tuning or sensitivity between responses in the two species. The lowest thresholds for sound were at approx. 70 dB SPL and for vibrations approx. 0.01 cm/s².

Examples of saccular fiber responses from *R. p. pipiens* are shown in Fig. 6 and from *L. albilabris* in Fig. 7. The fiber in Fig. 6 had a BF at 70 Hz and shows very sharp phase-locking. It is relatively insensitive to vibrations with threshold around 0.25 cm/s². Most notably, however, it only responds to very high sound levels with a threshold of 85 dB SPL. The *L. albilabris* fiber shows generally the same response: it is slightly more sensitive

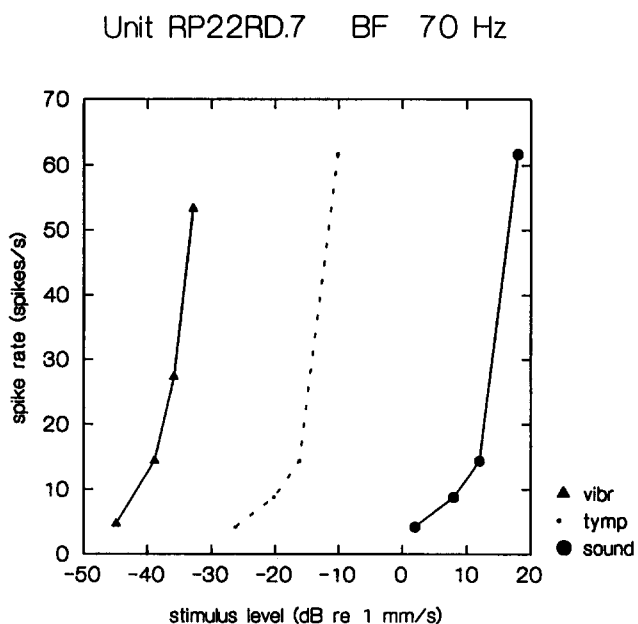


Fig. 8. Rate-level curves for a saccular fiber from *R. p. pipiens* stimulated with sound (circles) and vibration (triangles) at its BF (70 Hz). Other details same as Fig. 4

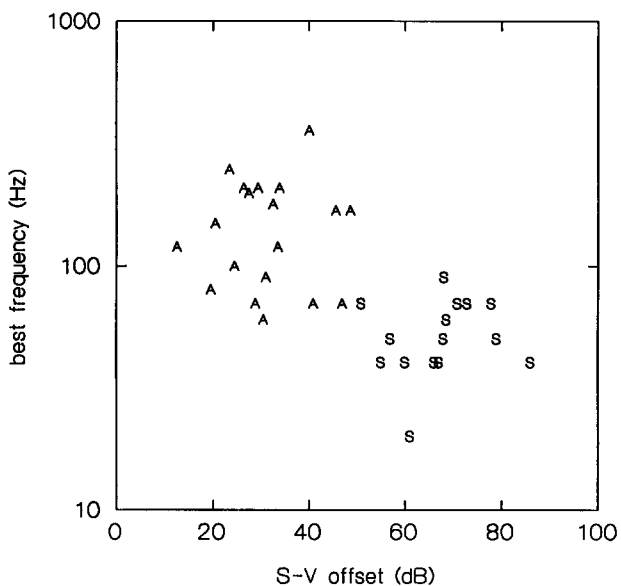


Fig. 9. Scatterplot of S-V offsets and BFs in 38 VIIIth nerve fibers in both species. Elements in the “A” and “S” cluster were partitioned according to their S-V offset using a K-means clustering algorithm (Systat, v. 5.1). See text for further details

with threshold at approximately 0.05 cm/s^2 . However, it is relatively insensitive to sound with a threshold of 90 dB SPL.

The rate-level curves for the saccular fibers (Fig. 8) are similar for sound and vibration stimulation. However, the S-V offsets are characteristically larger than in the AP-fibers; here the S-V offset is 50 dB and it generally ranges from 50 to 80 dB for the 39 saccular fibers.

BF and S-V offset is plotted in Fig. 9 for the 38 AP and saccular fibers in both frog species for which we have

sufficient data for both sound and vibration. The data were subjected to K-means clustering (Systat, v. 5.1) based on the S-V offset and partitioned in the two sets (“A” and “S” in the figure). In all instances, units classified as AP-units based on their location in the nerve fell within the “A” cluster, whereas saccular units fell in the “S” cluster. The means of the two clusters were found to be significantly different ($P < 0.001$) using the *t*-test. Thus, it appears that the S-V offset can be used as a reliable criterion to decide whether a unit originates in the AP or in the sacculus. The figure also shows that the BF ranges of the saccular and the AP fibers overlap.

Discussion

This study shows that many low-frequency AP-fibers of *R. pipiens pipiens* and *L. albilabris* exhibit remarkable sensitivity to vibrations. All AP-fibers with BFs below 4–500 Hz were sensitive to both sound and vibration. Similarly, all saccular vibration-sensitive fibers could also be stimulated by sound, albeit only at relatively high intensities. Moffat and Capranica (1976) reported a comparable sound sensitivity in saccular fibers of *Bufo americanus* and recently Yu et al. (1991) have reported a similar dual sensitivity in VIIIth nerve fibers in *Rana catesbeiana*. However, Yu et al. find a difference in the BFs of AP and saccular fibers: their AP fibers have BFs above 100 Hz, whereas the saccular fibers have BFs ranging from 40 to 80 Hz, so the frequency ranges of the two organs are separated. In contrast, our AP fibers have BFs down to 60 Hz and thus overlap the frequency range of the saccular fibers considerably (see Fig. 9). For AP fibers, BFs down to 10 Hz for vibrations have been found in *R. temporaria* (Jørgensen and Christensen-Dalsgaard 1991) and BFs as low as 60 Hz for sound have recently been described in *R. p. pipiens* (Schmitz et al. 1992).

Not only do the frequency ranges of the AP and saccular fibers overlap, but so do the ranges of sensitivities, AP fibers having as high vibration sensitivity as the saccular fibers. The vibration thresholds of the AP fibers range from 0.01 cm/s^2 to 1 cm/s^2 . The vibration thresholds of the saccular fibers range from 0.01 cm/s^2 to above 1 cm/s^2 . Jørgensen and Christensen-Dalsgaard (1989, 1991) also found that in *R. temporaria*, fibers in the posterior branch of the VIIIth nerve (probably innervating the AP) were nearly as sensitive to vibrations as the saccular fibers: sensitivities (spike rate thresholds) of posterior branch fibers ranged from 0.04 cm/s^2 , whereas those of saccular fibers ranged from 0.02 cm/s^2 . Similarly, Yu et al. (1991) report that AP and saccular fibers in *R. catesbeiana* have nearly comparable vibration sensitivities.

The similarity in the form of the rate-level curves of the fibers’ response to sound and vibration may indicate that sound and vibration stimulate the sensory cells in similar ways, producing similar movements of the endolymphatic fluid. The transmission loss from sound in air to vibration of the eardrum may account for a part of the S/V offset; however, when the vibration amplitude of the eardrum is estimated it is noteworthy that the

fibers in all cases are more sensitive to dorso-ventral vibration than to vibration of the tympanum (see Figs. 4 and 5). If the amplitude of endolymphatic movement caused by tympanal vibration is similar to that caused by dorso-ventral vibration, apparently the orientation of the AP sensory cells favors reception of dorso-ventral vibration. This observation suggests that tympanal vibration is not the main input for these cells (see below).

From this study, it appears unlikely that fibers innervating the utricle are vibration-sensitive, in contrast with the suggestion by Jørgensen and Christensen-Dalsgaard (1991). These authors suspected that some of their vibration responses originated in the utricle, because the distribution of BF's in the anterior branch of the VIIIth nerve was different from that in the saccular nerve. However, in this study we recorded from the utricular branchlet and found no vibration-sensitive fibers. In contrast, we found fibers with BF's ranging from 10 to 80 Hz in the saccular nerve, i.e. covering the range of BF's that Jørgensen and Christensen-Dalsgaard found in the anterior branch.

Both AP and saccular fibers show remarkable phase-locking at low frequencies, as it has been found previously in frog VIIIth nerve fibers (Narins and Hillery 1983; Narins and Lewis 1984; Christensen-Dalsgaard and Jørgensen 1988). Phase-locking enables the fibers to encode temporal parameters very accurately. Hypothetically, units in the CNS could also perform frequency analysis based on the temporal pattern of discharges, i.e. the interspike intervals, of the VIIIth nerve fibers. However, as our results show, to accurately determine frequencies the central units would need to collect inputs from several peripheral units, since the fibers discharge once per cycle only at low frequencies and high stimulus intensities. For example, fibers with BF's at 100–200 Hz (Fig. 3) may only discharge every 3rd to 5th cycle, and their discharge pattern would thus constitute a rather inexact representation of the stimulus frequency.

We find no difference in frequency ranges, sensitivities, phase locking or S–V offset between *R. p. pipiens* and *L. albilabris*. Narins and Lewis (1984) found sensitivities to accelerations of 0.001 cm/s² in *L. albilabris*, i.e. about 20 dB higher sensitivities than in the present study. However, these results were based on synchronization criteria. From the study of Christensen-Dalsgaard and Jørgensen (1988) we would expect sensitivities measured as spike-rate thresholds to be up to 20 dB lower, since the fibers respond to low-level accelerations by phase locking without increasing the mean spike rate and only increase the mean spike rate at higher stimulus levels. Furthermore, in the earlier study of *L. albilabris* (Narins and Lewis 1984), the animals were anesthetized topically and immobilized, whereas the animals in the present study (as in all other previous studies on frog vibration sensitivity) were anesthetized with nembutal, which is known to depress spontaneous activity in the frog auditory nerve (Feng et al. 1975). Therefore, the sensitivities reported here are probably comparable to those reported earlier suggesting that all frog species studied to date possess comparable vibration sensitivities.

It was earlier reported that BF's of vibration-sensitive fibers in *L. albilabris* covered the frequency range of 20 to 300 Hz (Narins and Lewis 1984). All these fibers were assumed to be saccular afferents. However, since the recordings were made in the VIIIth nerve it was impossible to unambiguously determine the origin of the fibers studied. Indeed, given the present results, it is most likely that the fibers with BF's in the high end of the frequency range were AP afferents. Consequently, the frequency range of BF's of the saccular fibers in the investigated frog species is apparently similar, generally ranging from 10 to 80 Hz.

Apparently, the special use of vibrational communication in *L. albilabris* is not reflected in the response characteristics of its VIIIth nerve fibers (although we of course cannot exclude the possibility that our relatively small sample of fibers in *L. albilabris* has missed a population of sensitive fibers). Instead, the general high vibration sensitivity of all the frog species investigated so far (which is also suggested by the prominence of the sacculus in all anurans) may point to a general behavioral use of vibration detection for other purposes than communication. For example, it is likely that the vibration sense can be used to alert frogs to predators, resulting in the familiar observation that anurans stop calling in response to light footfalls (Lewis and Narins 1985; Lewis and Lombard 1988). As a “burglar alarm”, the vibration sense could give the frog an early warning of approaching predators. Of course, such a sensitive sensory system could then secondarily be used for vibrational communication, as in *L. albilabris*. We would expect that specializations for vibrational communication could be found at higher stations in the CNS, for example involving processing of the temporal structure of the vibrational signals. Central processing of vibrational stimuli has not been studied in *L. albilabris*, but in *R. temporaria* Christensen-Dalsgaard and Jørgensen (1989) recorded responses of vibration-sensitive neurons in the midbrain that encoded the onset and duration of vibration pulses.

For several years it has puzzled researchers that many fibers in the VIIIth nerve have BF's below 300 Hz where the eardrum is a poor sound receiver. Lombard and Straughan (1974) and Wilczynski et al. (1987) showed that low-frequency fibers in *R. p. pipiens* would respond to free field sound even when both eardrums were removed or occluded. They were unable to identify the mechanism of this “extratympanic” input to the inner ear, but showed that the extratympanic input was most efficient at frequencies below 300 Hz. At these frequencies the AP fibers would also be very sensitive to vibrations and, in fact, more sensitive to dorsoventral vibrations than to vibrations of the eardrum (Figs. 5 and 6). Thus, the vibration sensitivity of the AP fibers could contribute to their extratympanic sensitivity, if free-field sound is somehow converted to vibrations of the frog's body. Several mechanisms can be envisioned for such a conversion: One mechanism is that free-field sound induces vibrations in the substrate. The efficiency of this pathway depends on the structure of the substrate: light substrates (e.g. leaves or branches) will vibrate more in the sound field than more massive substrates as soil.

Thus, for some species this pathway might be behaviorally important. Experiments where frogs are stimulated with low-frequency free-field sound can be difficult to interpret, since our (unpublished) pilot experiments show that sound-induced vibrations in a setup can be considerable: at sound levels of approx. 80 dB SPL we measured vibration levels from a cork platform in a free field setup of 3–7 cm/s² (i.e. more than 40 dB above the thresholds of the most sensitive units) in the frequency ranges of 100 to 400 Hz.

Two other mechanisms for reception of sound via the “extratympanic” pathway are 1) that sound is transmitted to the inner ear via bone conduction, i.e. sound-induced vibrations of the skull and 2) that sound induces pulsations of the frog’s lungs that might be transmitted to the inner ear via endolymphatic pathways (Narins et al. 1988). Recent measurements of the sound-induced vibrations of the body wall suggest that the lung vibrates as a simple resonator in the sound field (Ehret et al. 1990; Jørgensen et al. 1991; Jørgensen 1991). Thus, the frequency response of the lung depends on the lung volume (and hence on the size of the frog). Jørgensen (1991) measured body wall vibrations in four different anuran species and found maximal vibrations at frequencies ranging from 0.7 kHz in *R. temporaria* to 1.5 kHz in *Hyla versicolor*. At frequencies below 400 Hz the body wall vibration of all species is reduced considerably. However, the vibrations induced by the lung pulsations may still be above the vibration thresholds of some of the AP-fibers. Thus, even at the low frequencies the “lung input” may contribute to the extratympanic sensitivity.

However, the possibility remains that the low-frequency AP-fibers are vibration detectors that mainly respond to substrate-borne vibrations. The fibers would then complement the saccular fibers that have similar sensitivities, but over a more restricted frequency range, thus enabling the AP-fibers to subservise a different behavioral role. A use of low-frequency auditory fibers for detection of substrate-borne vibrations has been suggested earlier for mole-rats, especially of the genus *Spalax*, where the low-frequency region of the cochlea is enlarged and the anatomy of skull and middle ear show special adaptations that probably facilitate reception of substrate-borne vibrations (Rado et al. 1989). Several mole-rat species have been reported to use vibrational signals in intraspecific communication (Rado et al. 1987; Narins et al. 1992). Also, Hartline (1971) found that in snakes columella vibration and head vibration resulted in similar stimulation of the auditory nerve and suggested that an important biological function of the snake ear is to detect substrate-borne vibrations.

In conclusion, low frequency sound and vibrations do not appear to be distinct modalities in the frog. This enables the frog’s auditory system to receive sound-induced vibrations from the substrate or to respond to substrate-borne vibrations, and, conversely, enables the saccule to respond to (intense) airborne sound. It remains to be seen how (if at all) the frog can distinguish low-frequency airborne sound from low-frequency substrate-borne vibrations. A possible method would be that the CNS compares the excitation of saccular and AP fibers,

since airborne sound predominantly excites the AP fibers, whereas substrate-borne vibrations excite both types of fibers. However, the existence of extratympanic sound reception, either via bone conduction or via induced vibrations in the substrate makes the distinction between airborne sound and vibration even more difficult and should caution us that such a distinction might not be behaviorally important.

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