The vertebrate ear as an exquisite seismic sensor

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The neotropical frog *Leptodactylus albilabris* exhibits the greatest sensitivity to substrate-borne vibrations (seismic stimuli) reported to date for any terrestrial animal. Nerve fibers from the source of this extraordinary sensitivity in the ear show clear stimulus-evoked modulations of their resting discharge rates in response to sinusoidal seismic stimuli with peak accelerations less than 0.001 cm/s² (10^{-6} g). Evidence indicates that its source is the saccule, an organ of hearing in fish and of balance in man. We report that single vibration-sensitive fibers in the white-lipped frog saturate at (whole animal) displacements of 10 Å peak to peak [Fig. 1(b)]. Assuming a conservative 20-dB dynamic range for these fibers, the *in vivo* frog saccule and the mammalian cochlea exhibit roughly equal sensitivities to displacement.

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INTRODUCTION

Sensitivity to substrate-borne vibrations has been demonstrated in the inner ears of several species of anuran and caudate amphibians (Ashcroft and Hallpike, 1934; Smith, 1968; Ross and Smith, 1980; Caston *et al.*, 1977; Ratkinova, 1980; Koyama *et al.*, 1982). Among these, only in the North American bullfrog *Rana catesbeiana* has the seismic sensitivity been quantified and clearly distinguished from auditory sensitivity (Koyama *et al.*, 1982; Lewis, 1983). The most sensitive seismic afferents in that animal were shown to originate at the saccule and to exhibit identifiable responses to vibrational sinusoids with peak accelerations in the neighborhood of 0.02 cm/s² (Koyama *et al.*, 1982). The only animals demonstrated to have comparable seismic sensitivities had been snakes (Hartline, 1971) and the cockroach *Periplanata americana* (Autrum and Schneider, 1948), both of which were reported to have thresholds of 0.02 cm/s² for vibrational stimuli.

The white-lipped frog, *Leptodactylus albilabris* (Gunther) inhabits the Luquillo Mountains and adjacent lowlands of Puerto Rico. Nocturnally active, males of this species frequently call from the edges of accumulated ground water while partially embedded in the muddy substrate or stationed under dead *Cecropia* (sp.) leaves. We have found that such males conspicuously alter their calling patterns in response to very light, remote human footfalls or very light tapping of the ground with a finger, suggesting that these animals are good candidates for truly extraordinary seismic sensitivity. We report here the results of direct tests of that suggestion.

I. METHODS

Calling male *L. albilabris* were captured, transported to the laboratory, and held in conventional terrariums. In preparation for physiological studies, each animal was immobilized with d-tubocurarine chloride (E.R.) Squibb; 3 μl/g body weight; 3 mg/ml. After a topical anesthetic (lidocaine) was applied to the roof of the mouth, the VIIIth cranial

nerve trunk was exposed. The animal was turned ventral side up and rigidly secured to an aluminum plate which was mounted on a Bruel and Kjaer 4809 permanent magnet vibration excitor. Vibration amplitudes were monitored by a Bruel and Kjaer 8306 accelerometer affixed to the plate with
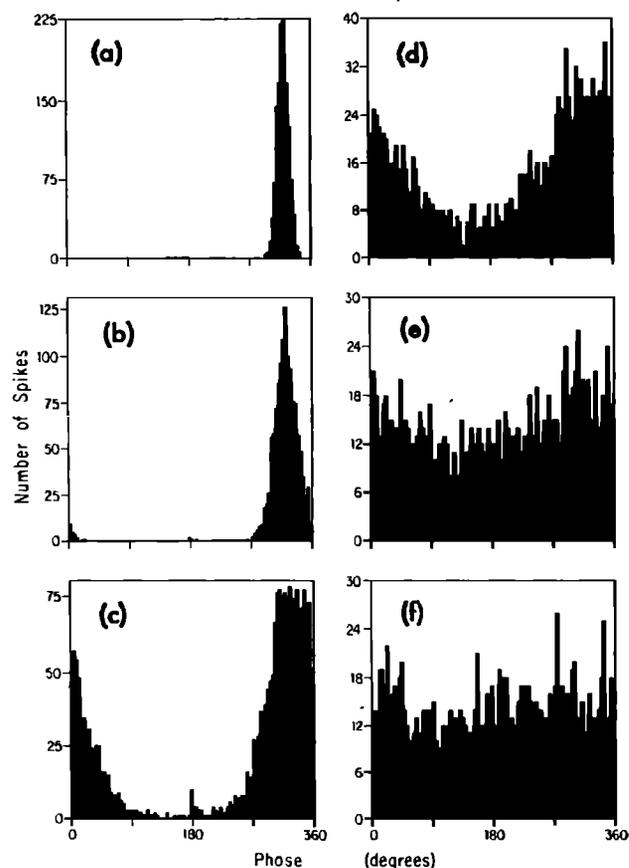

FIG. 1. Phase histograms showing response of a single seismic afferent fiber (040782-2) in the VIIIth nerve of *L. albilabris* to a series of vibratory stimuli at the fiber's BSF of 130 Hz for decreasing peak accelerations: (a) 0.2 cm/s², (b) 0.063 cm/s², (c) 0.02 cm/s², (d) 0.0063 cm/s², (e) 0.002 cm/s², (f) 0.00063 cm/s². Horizontal axis is the phase of the stimulus sinusoid; vertical axis is the number of spikes in a 3.6° (1%) phase bin. All histograms were obtained by accumulating the fiber's output for 20 s, except (a) and (b) (10 s).

beeswax. The physiological state of the animal was monitored periodically by observing capillary blood flow in the iris and arterial blood color. The preparation was placed on a five-stage (approximately 1000 kg) vibration-isolation table enclosed in an acoustic-isolation chamber (Koyama *et al.*, 1982) For frequencies from 10–1000 Hz, the spectral density of vibrational noise at the aluminum plate was below the noise floor (0.0002 cm/s^2 per root Hz) of the monitoring system.

Applying sinusoidal vibrations along the dorso-ventral axis of the animal, with the peak amplitude of the stimulus fixed at 0.5 cm/s^2 and the frequency swept from 20–350 Hz, we probed individual fibers in the anterior region of the VIIIth nerve with KCl-filled glass microelectrodes. Most of the penetrated fibers exhibited no discernible response at all to the stimulus. However, in ten individual male frogs we found 44 fibers with conspicuous response over part of the test-frequency range, exhibiting one or two spikes rigidly phase locked to each stimulus cycle. In every case, the response continued as the stimulus intensity was reduced to levels below 0.01 cm/s^2, and in a few cases to levels as low as 0.002 cm/s^2 [see Fig. 1(d) and (e)].

The possibility that these responses were mediated by auditory rather than vibratory pathways was ruled out as follows: with the peak amplitude of the vibratory sinusoids fixed at 7 cm/s^2, the resulting open-field sound intensity was measured at the level of the frog's tympanum with a calibrated condenser microphone (Bruel and Kjaer 4186). It was found to be less than 40 dB SPL over almost the entire frequency range (10–300 Hz) of observed seismic sensitivity, the exception being the 140- to 160-Hz range, where it approached 60 dB SPL. By linear extrapolation, the open-field sound intensity produced by 0.1 cm/s^2 vibrations was close to 0 dB SPL over most of the 10 to 300-Hz frequency range, approaching 20 dB SPL in the 140- to 160-Hz range. Spot checks of sensitivity to closed-field auditory stimuli among the seismic fibers indicated thresholds in the vicinity of 80 dB SPL. Thus at the stimulus levels of concern to us, the auditory-stimulus side effects of our seismic stimuli were negligible. Indeed, they were far below the lowest stimulus thresholds reported for primary auditory fibers in frogs (Capranica, 1976; Lewis, 1983; Lewis *et al.*, 1982).

II. RESULTS AND DISCUSSION

Among the 44 seismic fibers, 37 showed maximum sensitivity to vibratory stimuli at a single, well-defined "best seismic frequency" or BSF, whereas seven fibers were either broadly tuned or showed multiple sensitivity peaks. A fiber's BSF was determined manually by adjusting the frequency of a nonsaturating seismic stimulus for maximum response (spike rate). Typically the unit was analyzed at the BSF determined in this way, at the BSF ± 10 Hz, and at the BSF ± 20 Hz, time allowing. Based on their BSFs, the 37 fibers with single tuning peaks may be grouped into two classes. The first class ($N = 26$) consists of extremely sensitive, low-frequency units with BSFs between 20 and 160 Hz, and the second class ($N = 11$) is made up of less sensitive fibers with BSFs between 220 and 300 Hz (Lewis and Narins, 1984). Figure 1 shows the responses of a representative low-

frequency fiber to sinusoidal vibrations at the fiber's BSF (130 Hz) for six different peak accelerations. In response to the higher accelerations used, the fiber's firing rate reflects clipped and otherwise highly nonlinear responses to the stimulus sinusoid [Fig. 1(a)–(c)]. As the stimulus acceleration is decreased, the firing rate reflects predominantly linear responses [Fig. 1(d)–(f)], enabling one to calculate a linear gain. This in turn allows objective comparison (transcending definitions of threshold or "clear response") of sensitivities in different species. For example, the phase histograms in Fig. 1(d) and (e) reflect a linear gain factor of approximately 10 000 spikes/s per cm/s^2, nearly an order of magnitude greater than the linear gain of the most sensitive seismic afferents reported from the North American bullfrog (Koyama *et al.*, 1982; Lewis, 1983).

Figure 2(a) shows the response of another low-frequency fiber (BSF = 90 Hz) in which the peak acceleration was held constant at 0.5 cm/s^2 while its frequency was logarithmically swept at 8 oct/min between 30 and 200 Hz. This stimulus drove the fiber into its nonlinear operating range, resulting in a saturation of the fiber's per-cycle discharge rate over a frequency range of nearly three octaves [Fig. 2(a)]. However, within this range the phase-locking precision to the sinusoidal acceleration stimulus is strongly frequency dependent [Fig. 2(b)]. The measure we used to quantify the phase-locking precision of a fiber is the standard deviation σ of the phase about the preferred phase with respect to the stimulus sinusoid[1] (Koyama *et al.*, 1982). Low values of σ are indicative of strong phase locking, whereas high values of σ reflect weak phase locking. Figure 2 illustrates that although this fiber appears saturated in terms of its per-cycle dis-
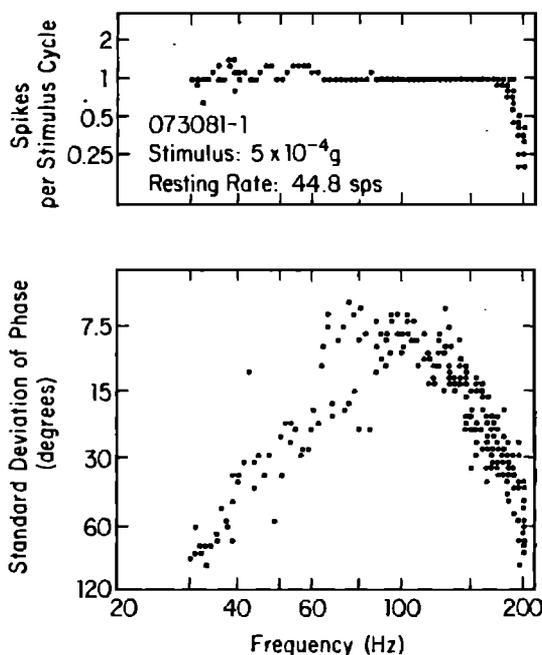

FIG. 2. (a) Per-cycle firing rate as a function of frequency for a seismic afferent fiber (073081-1) with a BSF of 90 Hz. Stimulus was a constant-acceleration (0.5 cm/s^2), sinusoidally varying displacement which was logarithmically swept at 8 oct/min from low to high frequencies. (b) Standard deviation of the phase of the response referred to the stimulus sinusoid as a function of frequency for the same cell as in (a). The resting discharge rate of this fiber was 44.8 spikes/s.

charge rate over a relatively wide frequency range, it nevertheless reflects frequency selectivity in terms of the degree to which it phase locks to the stimulus. This was typical of the low-frequency fibers that we observed; they showed a remarkable degree of "phase-lock tuning," with the strongest phase locking at the unit's BSF. The CNS therefore might derive stimulus frequency information from the degree of spike synchrony among several fibers with similar BSFs. On the other hand, it could obtain such information directly from the periodicity of the spike train in a single fiber.

Figure 3(a) shows a phase histogram of the responses of a typical high-frequency sensitive fiber. Note the greater spread of response spikes (standard deviation of the phase), compared to that of a low-frequency fiber stimulated with the same accelerations [Fig. 1(b)]. When subjected to a continuously swept acceleration of 0.2 cm/s^2, this fiber exhibited rather remarkable tuning in its per-cycle firing rate [Fig. 3(b)]. Similar sharply tuned responses have been observed in primary seismic afferents in the bullfrog saccule (Lewis, unpublished data). For this reason we believe that the responses we have recorded to seismic stimuli originate in the saccule in the inner ear. Dye filling individual axons and subsequent tracing of these fibers to their source will unequivocally identify the anatomical origin of the observed seismic responses.
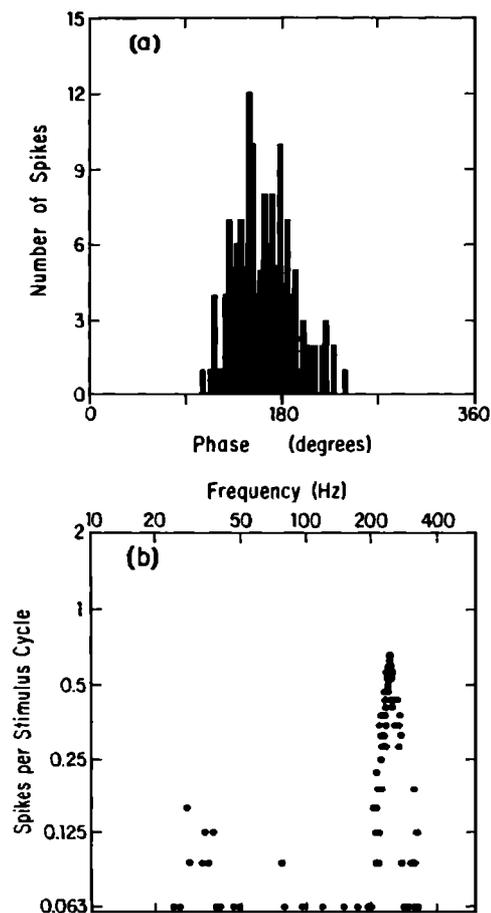

Phase   (degrees)

Frequency (Hz)

FIG. 3. (a) Phase histogram of responses of a single seismic afferent (040882-4) to 17 s of sinusoidal displacement with peak accelerations of 0.05 cm/s^2 at the unit's BSF (275 Hz). Bin width: 3.6°. (b) Mean per-cycle firing rate, averaged over 32 consecutive cycles as a function of frequency, obtained by sweeping a constant-acceleration sinusoid (0.2 cm/s^2) across the unit's response range at 8 oct/min.

To investigate the anatomical substrate which may underlie the remarkable physiological seismic sensitivity of *L. albilabris*, we have made preliminary observations of the sensory macula of the saccule under the SEM (Narins and Lewis, 1983). The saccular macula is roughly kidney shaped as it is in the bullfrog *R. catesbeiana*, with a predominance of hair cells characterized by a series of graded-length stereocilia and a single, eccentrically placed, bulbed kinocilium (type D hair cell *sensu*, Lewis and Nemanic, 1972). The bulbed kinocilia are connected directly to a thin pad of dense gelatinous material (the otoconial membrane). The other side of this membrane is connected in some unknown way to a viscous slurry of otoconia, occupying a very large proportion of the inner ear volume. On its macular side, the otoconial membrane is connected to the supporting cells by a dense fibrous mat.

It has been shown that saccular hair cell sensitivity is roughly proportional to the number of stereocilia per hair cell (Hudspeth and Jacobs, 1979). However, stereocilia densities in the white-lipped frog saccule (about 30–50 per hair cell), are in the same range as those found in the American bullfrog (Lewis and Li, 1975). In addition, our evidence to date (tuning curves and transient responses to seismic tone bursts) indicates that the mechnical network is low Q. Moreover, we have not observed any morphological adaptations in the saccule for increasing sensitivity to substrate vibrations (e.g., mechanical transformers involving levers).

It is of considerable interest that measurements of hair cell displacement sensitivity in the excised bullfrog saccule have revealed peak sensitivities of 20 mV/μm^{-1}; saturation of the receptor potential occurred for stereocilia displacements of 1.5 μ (Hudspeth and Corey, 1977). In contrast, we observe discharge rate *saturation* of seismic fibers in response to peak-to-peak (whole animal) displacements of 10 Å. This represents an *in vivo* sensitivity four orders of magnitude greater than the *in vitro* preparation. Perhaps the remarkable sensitivity to seismic vibrations in this animal has its anatomical basis in the interaction of the hair cells with the overlying otoconial membrane, or in the number of hair cells innervated by individual afferent axons.

ACKNOWLEDGMENTS

[1]Other common methods of quantifying phase locking are the coefficient of synchronization (Rose *et al.*, 1967) and the vector strength (Goldberg and Brown, 1969).

Ashcroft, W., and Hallpike, C. S. (1934). "Action potentials in the saccular nerve of the frog," J. Physiol. 81, 23P–24P.
Autrum, H., and Schneider, W. (1948). "Vergleichende Untersuchungen

über den Erschütterungssinn der Insekten," Z. Vgl. Physiol. 31, 77–88.

Capranica, R. R. (1976). "Morphology and Physiology of the Auditory System," in *Frog Neurobiology*, edited by R. Llinas and W. Precht (Springer-Verlag, Berlin), pp. 551–575.

Caston, J., Precht, W., and Blanks, R. H. I. (1977). "Response characteristics of frog's lagena afferents to natural stimulation," J. Comp. Physiol. 118, 273–289.

Goldberg, J. M., and Brown, P. B. (1969). "Response of binaural neurons of dog superior olivary complex to dichotic tonal stimuli: some physiological mechanisms of sound localization," J. Neurophysiol. 32, 613–636.

Hartline, P. H. (1971). "Physiological basis for detection of sound and vibration in snakes," J. Exp. Biol. 54, 349–371.

Hudspeth, A. J., and Corey, D. P. (1977). "Sensitivity, polarity and conductance change in the response of vertebrate hair cells to controlled mechanical stimuli," Proc. Natl. Acad. Sci. U. S. A. 74, 2407–2411.

Hudspeth, A. J., and Jacobs, R. (1979). "Stereocilia mediate transduction in vertebrate hair cells," Proc. Natl. Acad. Sci. U. S. A. 76, 1506–1509.

Koyama, H., Lewis, E. R., Leverenz, E. L., and Baird, R. A. (1982). "Acute seismic sensitivity in the bullfrog ear," Brain Res. 250, 168–172.

Lewis, E. R. (1983). "Dual acoustical sensitivity in frogs," in *Hearing–Physiological Bases, Psychophysics and Behavioural Studies*, edited by R. Klinke and R. Hatmann (Springer-Verlag, Berlin), pp. 61–66.

Lewis, E. R., Baird, R. A., Leverenz, E. L., and Koyama, H. (1982). "Inner ear: Dye injection reveals peripheral origins of specific sensitivities," Science 215, 1641–1643.

Lewis, E. R., and Li, C. W. (1975). "Hair cell types and distributions in the otolithic and auditory organs of the bullfrog," Brain Res. 83, 35–50.

Lewis, E. R., and Narins, P. M. (1984). "Do frogs communicate with seismic signals?," Science, in press.

Lewis, E. R., and Nemanic, P. (1972). "Scanning electron microscope observations of saccular ultrastructure in the mudpuppy (*Necturus maculosus*)," Z. Zellforsch. 123, 441–457.

Narins, P. M., and Lewis, E. R. (1983). "Thresholds and tuning characteristics for single vibratory fibers of the white-lipped frog," Abstr. ARO Res. Mtg. 6, 88–89.

Ratnikova, G. I. (1980). "Morphological and functional investigation of the saccular nerve in the frog *Rana temporaria*," Zh. Evol. Biokhim. Fiziol. 16, 60–65.

Rose, J. E., Brugge, J. F., Anderson, D. J., and Hind, J. E. (1967). "Phase-locked response to low frequency tones in single auditory nerve fibers of the squirrel monkey," J. Neurophysiol. 30, 769–793.

Ross, R. J., and Smith, J. J. B. (1980). "Detection of substrate vibrations by salamanders: Frequency sensitivity of the ear," Comp. Biochem. Physiol. 65A, 167–172.

Smith, J. J. B. (1968). "Hearing in terrestrial urodeles: A vibration-sensitive mechanism in the ear," J. Exp. Biol. 48, 191–205.