

## Acute seismic sensitivity in the bullfrog ear

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Single axons in the auditory/vestibular nerve of the American bullfrog exhibit by far the most exquisite sensitivity to substrate-borne vibration yet reported for a quadruped vertebrate. Earlier dye-injection studies revealed that these axons, which are relatively insensitive to airborne sound, originate at the saccular and lagenar maculae of the bullfrog inner ear. The more sensitive axons exhibited clear responses to vibratory sinusoids with peak accelerations as low as 0.005 cm/s<sup>2</sup>.

Using intracellular injection of the fluorescent dye Lucifer Yellow to identify the peripheral origins of specific sensitivities in the VIIIth cranial nerve of the American bullfrog, *Rana catesbeiana*, we recently confirmed the saccular and lagenar origins of seismic sensitivity (sensitivity to substrate-borne vibration) in that animal, demonstrating that seismic sensitivity resides over the entire saccular macula and over a narrow region at the center of the lagenar macula<sup>13</sup>. The presence of seismic sensitivity in the inner ears of frogs has been known for many years<sup>1,3,4,9,15</sup>. However, the magnitude of that sensitivity and its dependence on frequency for seismic sinusoids had not been determined. Before attempting to generate more-detailed maps of seismic sensitivity over the saccular macula and the center of the lagenar macula, we first required identification of the parameters of that sensitivity. Therefore, we set out to measure its magnitude and frequency dependence in single VIIIth-nerve axons in the bullfrog. What we found was by far the most exquisite seismic sensitivity yet reported for a quadruped vertebrate. Sixty percent of the seismic axons that we studied exhibited clear phase-locking of ongoing spike activity to

linear, sinusoidal substrate vibration with peak acceleration between 0.005 and 0.02 cm/s<sup>2</sup>. The only comparable linear acceleration threshold that we found reported previously was 0.02 cm/s<sup>2</sup> for snakes<sup>10</sup> and for the cockroach *Periplaneta americana*<sup>2,17</sup>. In the one reported example of mammalian inner-ear vibratory sensitivity, Young et al. reported thresholds greater than 0.1 cm/s<sup>2</sup> for single vestibular axons in the squirrel monkey<sup>20</sup>. The frequencies (approximately 15–200 Hz) covered by seismic sensitivity in the bullfrog extend well below those (100–2000 Hz) reported for snakes, the squirrel monkey, and most arthropods<sup>2,10,11,17,19,20</sup>. For frequencies above 3 Hz, ambient microseismic noise spectra exhibit nearly constant mean acceleration amplitudes (typically between 0.00001 and 0.001 cm/s<sup>2</sup>, depending on locale, over  $\frac{1}{3}$ -octave bands); the mean acceleration amplitudes increase sharply below 3 Hz<sup>6</sup>. Thus, the 15–200 Hz range is just high enough to avoid most of the typical ambient noise; at the same time it is sufficiently low to sense the vibrations produced by remote footfalls.

Although the floor of our laboratory is a concrete slab directly over bedrock, it nevertheless exhibits

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large seismic noise components at approximately 14 Hz (a building resonance), 60 Hz and 120 Hz (owing to 60 Hz electrical power in transformers, motors and the like throughout the building). Even quite massive platforms resting above the floor exhibit large-amplitude vibrations in direct response to airborne acoustical energy. Therefore, we conducted our experiments on a five-stage vibration filter table weighing more than 1000 kg and enclosed in an acoustical isolation chamber. With this system, all linear vibrations (vertical and horizontal) in the frequency range from 10–1000 Hz were reduced to levels below the noise floor of our measuring system (approximately  $0.0002 \text{ cm/s}^2$  peak acceleration).

Throughout the surgery and each experiment, the frog was anesthetized with sodium pentobarbital (approx.  $70 \mu\text{g}$  per g body weight). Through a small hole in the roof of the mouth, the VIIIth nerve was exposed in the cranial cavity medial to the intact otic capsule with intact circulation. The frog was mounted upside down on a rigid platform, with its head held firmly against the platform surface and its lower jaw held open to expose the access to the nerve. Dorsoventral sinusoidal seismic stimuli were generated by a Bruel and Kjaer (B and K) model 4809 exciter connected to the undersurface of the platform. The stimuli were monitored with an accelerometer (B and K model 4370 or 8306) mounted next to the frog's head on the platform. The loaded platform was calibrated with respect to spatial variation in vibration amplitude and was found to be uniform to within  $\pm 1 \text{ dB}$  over the entire frequency range of interest (10–300 Hz). Between 10 Hz and 200 Hz, coupling from platform vibration to air-borne sound was weak. The worst case was approximately 65 Hz, where a vibratory sinusoid with  $0.1 \text{ cm/s}^2$  peak acceleration produced 24 dB SPL (measured by linear extrapolation with a calibrated B and K 4166 condenser microphone) at the level of the frog's tympanum. 24 dB SPL is below the lowest thresholds (25–30 dB SPL) reported for bullfrog auditory afferent axons<sup>8</sup>. At other frequencies the coupling typically was 10 dB weaker (i.e., vibratory sinusoids with  $0.1 \text{ cm/s}^2$  peak accelerations produced 14 dB SPL at the tympanum). Spurious 60 Hz pickup in the leads to the vibration exciter was attenuated by means of a 40-dB resistive voltage divider at the exciter's input terminal; the resulting 60-Hz vi-

bration was below the noise floor of the measuring system.

To date we have used this system to study 46 seismic afferent axons from the bullfrog ear. On the basis of their activities in the absence of seismic stimuli, we divided those axons into two classes: (a) spontaneously-active axons, whose mean firing rates ranged approximately from 15 to 50 spikes/s; and (b) nearly-silent axons, whose mean firing rates were less than 7 spikes/s. Among the 46 axons, 28 were spontaneously active and 18 were nearly silent (of the latter, 11 had firing rates less than one spike/s). On the average, nearly silent axons were 15 to 20 dB less sensitive than were spontaneously-active axons. Axons of both classes were relatively insensitive to airborne sound, exhibiting thresholds greater than 80 dB SPL for closed-field auditory stimuli applied to the tympanum.

In the absence of seismic stimuli, spontaneously-active axons exhibited unimodal spike-interval histograms (Fig. 1). As the amplitude of a sinusoidal seismic stimulus was increased, the spikes of the spontaneously-active unit showed increasing tendency to be phase locked to the stimulus. As a result of this tendency, weak seismic stimuli typically left the mean firing rate of an axon virtually unchanged, yet drastically altered the spike-interval statistics, usually causing the histogram to become multimodal (Fig. 1). The phase-locking tendency also was made apparent by conventional phase statistics. The one we found most convenient for evaluation of

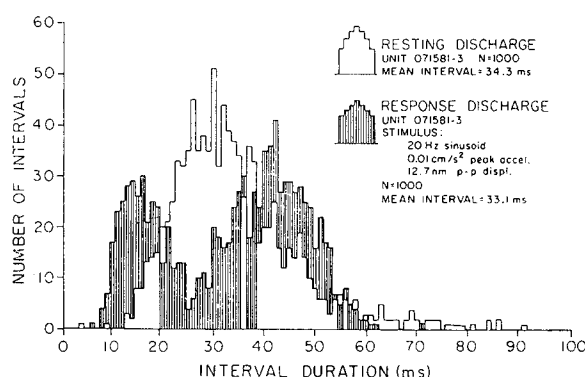


Fig. 1. Spike-interval histograms of a seismic axon whose best frequency was approximately 20 Hz. In response to a 20-Hz sinusoid with peak acceleration of  $0.01 \text{ cm/s}^2$ , the spikes of this axon tended to be phase-locked to the stimulus, with two spikes per stimulus cycle, leading to a bimodal interval distribution.

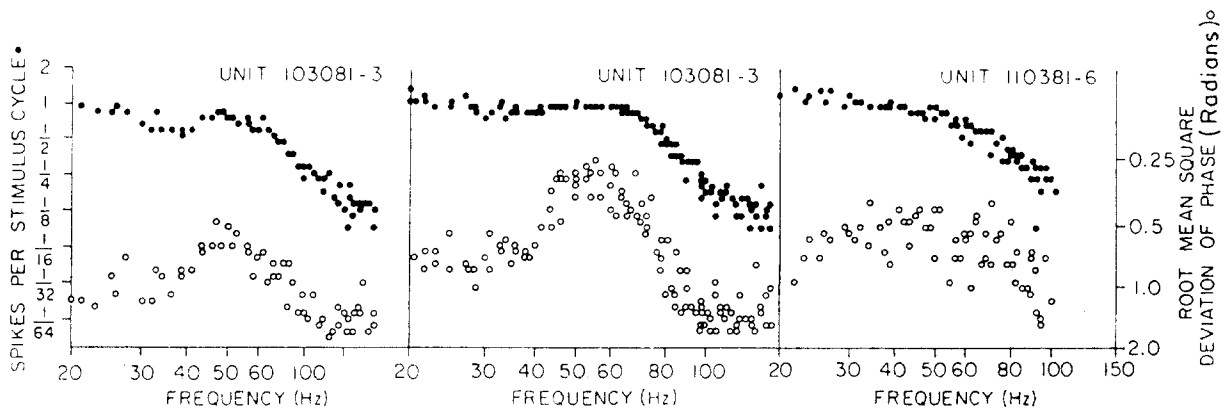


Fig. 2. Responses of a narrowly-tuned seismic axon (103081-3) and a broadly-tuned seismic axon (110381-6) to sinusoids of various frequencies. The scale on the right (for open circles) can be interpreted as follows: the value 1.81 corresponds to no phase preference at all for the first spike in each stimulus cycle: lower values indicate the existence of a preferred phase, and the value itself is the rms deviation of spike occurrence from that preferred phase (e.g. 0.25 corresponds to rms deviation of approximately  $15^\circ$ , which would be 0.8 ms at 50 Hz). Left: the spike rate of axon 103081-3 was barely saturated at one spike per cycle at the unit's best frequency (approx. 50 Hz). The stimulus level was  $0.2 \text{ cm/s}^2$  peak acceleration. Each dot represents the response averaged over 50 stimulus cycles. Center: responses of 103081-3 to stimulus level  $0.5 \text{ cm/s}^2$  peak acceleration. Each dot represents 50 stimulus cycles. Right: non-saturated responses of axon 110381-6 to seismic sinusoids with amplitude  $0.1 \text{ cm/s}^2$  peak acceleration. Each dot represents 20 stimulus cycles.

sensitivity bandwidth was the root-mean-square deviation of the phase (with respect to the stimulus sinusoid) of the first spike occurring during each stimulus cycle (Fig. 2). As the stimulus amplitude was increased to high levels (e.g., peak accelerations greater than  $0.1 \text{ cm/s}^2$ ), the firing rates of spontaneously-active axons became saturated, at one spike per stimulus cycle in the upper frequency range (typically between 50 and 120 Hz) and two spikes per cycle in the lower range (typically 15–40 Hz). Even after spike-rate saturation had occurred, the degree of phase locking continued to increase with increasing stimulus intensity (Fig. 2).

As the amplitude of a sinusoidal seismic stimulus was increased, the earliest sign of response in a nearly-silent axon was an increase in the mean spike rate. As the stimulus amplitude was increased further, the spike rate increased until it reached one or two spikes per cycle, depending on the stimulus frequency, at which level it saturated. The stimulus intensity required for saturation typically was greater than  $0.5 \text{ cm/s}^2$  (peak acceleration). The weakest sinusoidal stimuli eliciting any noticeable response in a nearly-silent axon typically had peak acceleration in the neighborhood of  $0.1 \text{ cm/s}^2$ . Clear responses typically could be observed in spontaneously-active axons to stimuli with peak accelerations in the range  $0.005\text{--}0.02 \text{ cm/s}^2$ .

The frequency bandwidth varied from axon to axon in both classes. Some axons exhibited broad tuning (e.g., Fig. 2, far right), with low-frequency corners between 20 and 50 Hz and high-frequency corners between 80 and 120 Hz. Other axons exhibited sharp tuning, with best frequencies ranging from approximately 20 Hz to approximately 150 Hz. The majority of the sharply-tuned axons exhibited best frequencies between 40 and 70 Hz.

Only one of the 46 axons in this study was injected with dye and traced to its peripheral origin. That axon belonged to the nearly-silent class and was traced to the saccular macula. In previous studies, we traced both classes of axon to both seismic endorgans (sacculus and lagena)<sup>13</sup>. However, we have not found a single axon that terminated on more than one endorgan. To date we have no evidence regarding any quantitative differences in the seismic sensitivities in the two endorgans, but we presume that such evidence will appear when we resume our dye-injection studies of those organs.

In his elegant study of seismic sensitivity in snakes, Hartline found convincing evidence that the most acute sensitivity is mediated by the auditory endorgan<sup>10</sup>. Indeed, he found that the auditory and acutely-sensitive seismic tuning curves were virtually identical. The less acute seismic sensitivity in snakes was somatic in origin. Crude seismic sensitivity has

been reported for the auditory endorgans (amphibian and basilar papillae) of frogs and salamanders<sup>7,8</sup>, where coupling between the substrate and the oval window apparently is provided by the opercular plate and the opercularis muscle<sup>14</sup>. However, it is unlikely that any of the 46 axons included in the present study originated at either of those organs. None of the 46 exhibited a tuning curve resembling that of a typical bullfrog auditory axon. In fact most of the 46 exhibited tuning peaks well below the bottom of the bullfrog auditory frequency range. Furthermore, approximately one-third of the 46 axons were penetrated in the VIIIth nerve's anterior branch lateral to its mergence with the posterior branch; the anterior branch does not innervate the auditory endorgans. Finally, in our earlier studies we identified acute seismic sensitivity in 25 afferent axons that subsequently were dye-filled and traced to their peripheral origins. All 25 originated either at the saccular macula or the lagenar macula, none at the auditory papillae. Therefore, the snake and the frog appear to have achieved their exquisite seismic sensitivities by different routes — the snake through modification of the reptilian auditory apparatus with its tectorial endorgan, the frog through retention and modification of the teleost auditory apparatus, with its otolithic/otoconial endorgans<sup>5</sup>. Ross and Smith recently have suggested that the latter route also was followed by salamanders<sup>16,18</sup>. The seismic thresholds that they reported for those animals, however, were in the neighborhood of 2 cm/s<sup>2</sup>, or approximately two orders of magnitude greater than stimulus levels eliciting clear responses in bullfrog seismic axons. Very recently, in collaboration with P. M. Narins, one of us (E.R.L.) has extended this work to the white-lipped frog, *Leptodactylus albilabris*, in which they found seismic axons, exhibiting clear phase-locking responses to sinusoidal vibrations with peak accelerations as low as 0.001 cm/s<sup>2</sup>.

A report on that work is in preparation.

As a final note, we wish to point out that over approximately one decade of frequency, the most effective seismic stimuli for the various axons required essentially the same minimum peak acceleration (0.005–0.02 cm/s<sup>2</sup>) in order to elicit clear responses in the bullfrog. Therefore we conclude that the endorgans involved truly are sensors of body acceleration. If they were sensors of body displacement, the required accelerations would have increased 100-fold as the frequency increased 10-fold. It seems clear from the studies of Hudspeth and Corey that the sensory cells (hair cells), at least those in the bullfrog sacculus, are displacement sensors<sup>12</sup>. Therefore we conclude that the otoconial membranes of the bullfrog sacculus and lagena possess transfer functions such that, in the frequency range of seismic sensitivity, linear acceleration of the frog's body is translated into linear displacement at the hair cell (stereocilia). Our estimates of displacement amplitudes at the stimulus levels just sufficient to produce clear response depend on our model of the micromechanics of the otoconial membrane system. If we assume that it is a low-Q mechanical system (e.g. critically damped or overdamped), then we could estimate the displacements from the highest frequencies at which the acute seismic sensitivity is found. For example, we have found clear responses to sinusoids at 100 Hz with peak acceleration amplitudes of 0.01 cm/s<sup>2</sup>, from which we would deduce hair-cell displacement amplitude equal to or less than 500 pm in a low-Q structure. Interestingly, 500 pm is the threshold estimated for bullfrog saccular hair cells by Hudspeth and Corey on the basis of a 10  $\mu$ V noise floor of the receptor potential<sup>12</sup>.

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