Do Male White-Lipped Frogs Use Seismic Signals for Intraspecific Communication?[1]

Edwin R. Lewis,[2,*] Peter M. Narins,† Kathryn A. Cortopassi,[3,‡]
Walter M. Yamada,[4,§] Eva H. Poinar,*‡ Steven W. Moore,[5,‡] and Xiao-long Yu[6,*]

*Department of EECS, University of California, Berkeley, California 94720-1771
†Department of Physiological Science, UCLA, Los Angeles, California 90095-1606
‡Graduate group in Bioengineering, University of California, Berkeley, CA 94720-1708
§Graduate group in Neuroscience, University of California, Berkeley, CA 94720-3200

SYNOPSIS. Modern frogs and toads possess a structurally unique saccule, endowing them with seismic sensitivity greater than that observed so far in any other group of terrestrial vertebrates. In synchrony with their advertisement calls, approximately half of the calling males of one frog species, the Puerto-Rican white-lipped frog (*Leptodactylus albilabris*), produce impulsive seismic signals (thumps). The spectral distribution of power in these seismic signals matches precisely the spectral sensitivity of the frog's saccule. The signals have sufficient amplitude to be sensed easily by the frog's saccule up to several meters from the source—well beyond the typical spacing when these frogs are calling in a group. This circumstantial evidence suggests that white-lipped frogs may use the seismic channel in intraspecific communication, possibly as an alternative to the airborne channel, which often is cluttered with noise and interference. Using the frog's vocalizations as our assay, we set out to test that proposition. In response to playback calls, the male white-lipped frog adjusts several of its own calling parameters. The most conspicuous of these involves call timing—specifically the tendency for a gap in the distribution of call onsets, precisely timed with respect to the onsets of the playback calls. When the airborne component is unavailable (*e.g.,* masked by noise), approximately one in five animals produces the calling gap in response to the seismic signals alone.

INTRODUCTION

The inner ears of frogs and toads (anurans) are known to be sensitive to both airborne sound and substrate borne vibration. Based on the connections of anatomical elements, Figure 1 shows putative pathways, into and through the inner ear of a typical modern frog or toad, for the microscopic structural (acoustic) displacements induced by these two modes of stimuli at the animal's periphery (see Lewis and Narins, 1998). The pectoral girdle is connected not only to the ground (*e.g.,* via the forelimbs) but also to the air (*e.g.,* via the body wall). The opercular system (comprising a skeletal element inserted into the oval window and a muscle connecting that element to the pectoral girdle) thus may conduct acoustic displacements from airborne sound as well as ground-borne vibrations (see Lombard and Straughan, 1974; Narins *et al.*, 1988). Whatever their peripheral sources might be, all pathways converge at the oval window. The length and diameter of the bypass channel (periotic duct) dictate that only a tiny fraction of the total acoustic displacement at the oval window is diverted through that channel. Almost all of it passes through the inner ear sensors.

Being the common element in all three

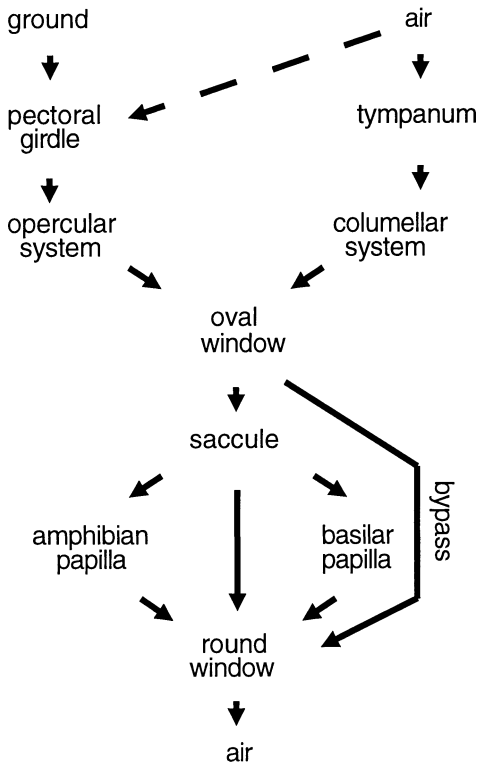## Putative acoustic displacement paths through the frog inner ear

FIG. 1. Putative paths by which acoustic waves flow into and through the ear of a frog. The columella is an analog of the mammalian ossicular chain.

sue core of the membranous labyrinth. Outside the thin epithelial wall is a volume filled with a sodium-rich aqueous solution (periotic fluid) and coupled directly to the oval window. In terrestrial vertebrates, otoconia are found in three inner-ear sensors, the saccule, the utricle, and the lagena. Each of these sensors is equipped with a pad of epithelial tissue (the macula) in which the mechanoreceptor cells (hair cells) are embedded and to which afferent and efferent axons project. In almost every case, the macula is embedded in thick connective tissue and appears to be coupled rigidly to the core of the membranous labyrinth. The one exception among terrestrial vertebrates is the saccular macula of the more recently derived frogs and toads, including *Rana catesbeiana* and *Leptodactylus albilabris*, the subjects of this paper. In those animals, the thin epithelial wall is extended and bounds the viscous suspension of otoconia ventrally and medially (in part) as well as laterally. The macular pad forms an island on the ventromedial part of the thin wall. On one side of the pad is the otoconial suspension; on the other side is periotic fluid (rather than thick connective tissue). In adult North American bullfrogs (*R. catesbeiana*), the saccular macula has approximately 2,500 hair cells (the number increases as the frog grows) and is innervated by approximately 1,100 axons (Lewis and Li, 1973; Dunn, 1978). It is interesting that this sensor, with its large investment in volume and its unique macular structure, is innervated by fewer axons than all but one of the seven other sensors in the bullfrog inner ear. The utricle, for example, has nearly four times as many axons; and the lagena has nearly twice as many. Only the basilar papilla has fewer.

When we first began to study the physiology of this organ in our laboratory at Berkeley, the fact that it was sensitive to vibration (*e.g.,* finger tapping) had been known for many years (Ashcroft and Hallpike, 1934; Cazin and Lannou, 1975; Gualttierotti, 1968; Ratnikova, 1980). Our early scanning electron microscope studies of hair-cell surface morphology in the otoconial and auditory organs (saccule, utricle, lagena, amphibian papilla and basilar papilla)

paths through the inner ear, the saccule is especially intriguing. Making the anuran saccule even more intriguing is the fact that the volume occupied by it is approximately equal to that occupied by all of the anuran inner ear's seven other sensory organs taken together. What the saccule evidently gives frogs and toads is exquisite sensitivity to substrate vibration, together with an extension of the range of general acoustic sensitivity to frequencies well below those covered by the two auditory papillae (amphibian and basilar).

Most of the anuran saccular volume is filled with a viscous suspension of calcium carbonate crystals (otoconia). The suspension is bounded on its lateral side by a very thin epithelial wall. On its medial side it is bounded in part by the thick connective-tis-
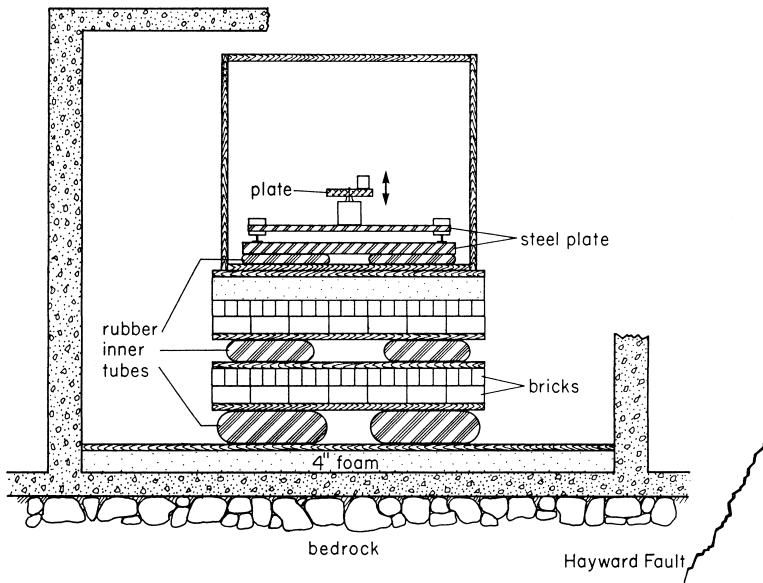
FIG. 2. Schematic diagram of the laboratory setup for studying seismic sensitivity in the frog ear. The frog was mounted on the (rectangular) plate, which in turn was mounted on the vibration exciter. Also mounted on the plate was a small piezoelectric accelerometer to measure the actual vibration stimulus delivered to the frog. In experiments with noise stimuli, the four corners of the plate were connected to adjustable visco-elastic elements, designed to reduce mechanical resonances and flatten the frequency response of the system.

of *R. catesbeiana* revealed six distinct types of hair bundles (Lewis and Li, 1975). The detailed maps of the distributions of these bundle types over the sensory maculae and papillae of the five organs strongly suggested corresponding functional distributions, including acoustic function for the saccule (*e.g.,* see Lewis, 1972; Lewis and Li, 1975). To test this hypothesis, by constructing precise functional overlays for our morphological maps, we undertook studies in which single, functionally-identified afferent axons of the frog eighth nerve were injected with dye and subsequently traced to the hair cells at which they originated (Lewis *et al.*, 1982). Two results were especially relevant to the saccule. (1) We found that all of the traditional vestibular functions (sensitivities to head orientation and to low-frequency motion of the head) were confined to the maculae of the utricle and lagena, and the cristae of the three semicircular canals. (2) All of the twenty-five axons that we traced to the saccule exhibited sensitivity to substrate vibration and, in some cases, airborne sound, but not to the traditional vestibular stimuli (Lewis

et al., 1982; Koyama *et al.*, 1982). Intrigued by the seismic component of saccular sensitivity, we undertook the first detailed study of its properties.

One of the first things that we had noticed about *R. catesbeiana* saccular afferent axons was a 60-Hz modulation of their spontaneous spike rates. The statistical distributions of their spike-intervals, for example, typically were multimodal—the modes being separated by approximately 17 msec. The floor of our laboratory (in the basement of Cory Hall) is a concrete slab directly on bedrock, and the ambient vibrations in that floor are extremely low. Nevertheless, either directly through the floor, directly through the air, or indirectly through sympathetic vibration of our experimental apparatus driven by airborne sound, 60-Hz energy was being delivered to our animal subjects. The sources of such energy (*e.g.,* 60-Hz synchronous motors and transformers) of course are numerous in any building in the USA. We achieved acoustic isolation by constructing a vibration filter table (depicted in Fig. 2) enclosed in a room with solid concrete walls and a

thick, gasket-sealed wooden door. The final two stages of the isolation table were enclosed in an inner chamber constructed of 3/4-inch plywood. The total mass of the vibration filter table was greater than 1,000 kg. With this arrangement, over the frequency range 10–1,000 Hz (including 60 Hz and its harmonics) all three ambient vibration components (one vertical, two horizontal) delivered to the animal were below the noise floor (approximately 0.000002 m/sec^2) of our measuring system.

We elected to use dorsoventral vibration as the stimulus. This was supplied to a thick plate from an electromagnetic exciter (Bruel and Kjaer model 4908). Although the electric leads to the exciter were doubly shielded, they nonetheless picked up energy at 60 Hz and its harmonics from ambient fields in the laboratory. Initially, the amplitudes of the resulting vibrations of the plate were kept below the noise floor of our measuring system by an attenuator inserted at the input terminals of the exciter. Subsequently, new equipment added to the neighboring laboratory generated intense 60-Hz magnetic fields. Acting directly on the exciter, even when its input terminals were short-circuited, these new fields produced unacceptable levels of 60-Hz vibration. Encasing the exciter in a thick mu-metal shell solved that problem, bringing the plate vibrations below our noise floor again. With frogs mounted on the plate, there was no visible 60-Hz modulation of spike rate (from spurious 60-Hz inputs) in any of the saccular axons that we studied. Our early experiments with this system were carried out with sinusoidal stimuli. In more recent experiments, we employed broadband white noise. Results with both stimuli are competely consistent with one another.

In our very first studies, we found that afferent axons of the *R. catesbeiana* saccule are exquisitely sensitive to dorsoventral vibrations, often exhibiting conspicuous responses for stimulus amplitudes as low as 0.00005 m/sec^2 (Koyama *et al.*, 1982). The only animals that had been demonstrated to have seismic sensitivity comparable to this were snakes (Hartline, 1971) and the American cockroach, *Periplaneta americana*, (Autrum and Schneider, 1948), both of

which were reported to have thresholds of 0.0002 m/sec^2. We also found that bullfrog saccular axons are well tuned for at least one aspect of the natural seismic environment; their tuning structures strongly reject energy in the frequency range (below 5 Hz) where microseismic noise in the ground becomes very large (Frantti *et al.*, 1962). Most are broadly tuned, with single pass bands (frequency ranges of maximum sensitivity) centered below 100 Hz, and with steep band edges on both the high- and low-frequency sides of the pass band.

In 1981, the study was extended to the Puerto Rican white-lipped frog, *Leptodactylus albilabris*, a ground-dwelling animal commonly found at the rain-forest sites used by P. M. Narins and colleagues for field studies of the arboreal Puerto Rican coqui frog, *Eleutherodactylus coqui*. Narins and his students had noticed that, in response to footfalls, even very gentle ones many meters away, isolated *L. albilabris* males often cease calling. Studies of these animals in our Berkeley laboratory revealed saccular afferent axons with measurable responses for dorsoventral vibration amplitudes as low as 0.0000063 m/sec^2 (Lewis and Narins, 1981; Narins and Lewis, 1984). The tuning of saccular axons in *L. albilabris* proved to be similar to that in *R. catesbeiana*. Most axons are broadly tuned, with pass bands centered below 100 Hz.

Assuming that the male frog was silenced by the seismic component of the footfall, we planned to carry out a simple experiment: using geophones placed close to calling males in the field, we would measure the amplitudes of the seismic stimuli required to cause them to stop calling. We never carried out that experiment. Having placed a geophone and a microphone approximately 1.0 m from our first subject in the field, we found that when the frog eventually recovered from the disturbance and began to call again, each of its airborne calls was accompanied by a conspicuous, precisely-synchronized seismic wave (Fig. 3, see Lewis and Narins, 1985). The airborne call (Fig. 4) comprised a short, lower-frequency tone burst (approximately 1.1 kHz), followed by a longer, higher-frequency burst (approximately 2.2 kHz). Spectral
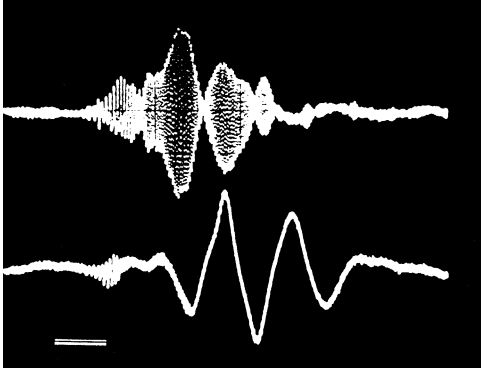
FIG. 3. Sound pressure waveform of the airborne call (upper trace) and vibrational velocity waveform of the vertical component of the accompanying seismic thump (lower trace) of a male white-lipped frog. Both were recorded at approximately 1 m from the source. The peak vertical acceleration of the thump, measured at 1.0 m, was approximately 5×10^{-4} g (1.0 g = 9.81 m/sec^2). Horizontal line = 10 msec.

analysis revealed that the power of the vertical component of the seismic wave was concentrated in the 20–70 Hz range. The tuning of the saccular afferent axons to dorsoventral vibrations is well matched to this spectrum.

In the habitat of *L. albilabris*, the air often is filled with the sound of the wind and the calls of other, mostly arboreal, frog species (especially *E. coqui*). We immediately were struck by the possibility that, under such circumstances, *L. albilabris* might use the relatively quiet seismic channel as an alternative. Therefore, we set out to obtain definitive evidence of involvement of the seismic wave in the male-male communication of that animal. In attempting to do so, we undertook what has evolved into a long series of behavioral studies in the field. These have involved artificial thumps, generated by a mechanical device (thumper), and have focused on a gap in the temporal distribution of the male frog's call onsets in the presence of calls of conspecific males. A constant problem in this study has been the inevitable presence of an airborne sound accompanying the seismic wave produced by the thumper. In this paper, we begin by illustrating some of the relevant results from physiological studies of both the *L. albilabris* and *R. catesbeiana* inner ears. Then we describe, in a general way, the
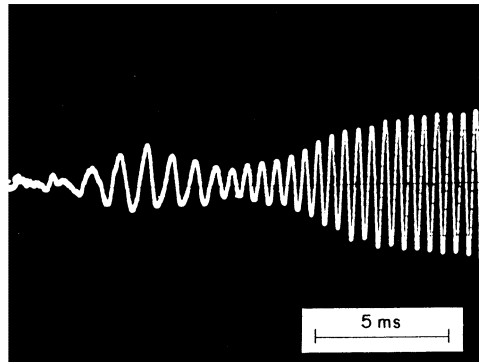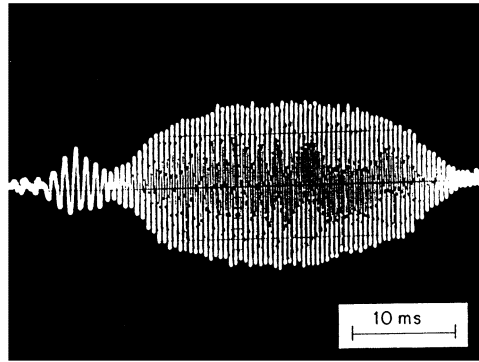

FIG. 4. Sound pressure waveform of the airborne call of a non-thumping male white-lipped frog.

methods we employed in the field and some of the results we have obtained.

REVIEW OF PHYSIOLOGICAL RESULTS

Physiological measurements were made by penetrating individual eighth-nerve afferent axons with microelectrodes and recording spike traffic on the way from the intact ear (otic capsule unopened) to the brainstem. Each axon, along with the hair cells it innervates and the peripheral tuning structures connected to those hair cells, is defined to be a sensory *unit*. Figure 5 shows the results, for one saccular unit, of a typical experiment with dorsoventral sinusoidal vibration applied directly to an *L. albilabris* subject. At each stimulus amplitude, several thousand cycles of the stimulus sine wave were applied, and the phase of the sine wave at the onset of each spike was determined. The histograms constructed from these data (phase histograms) show
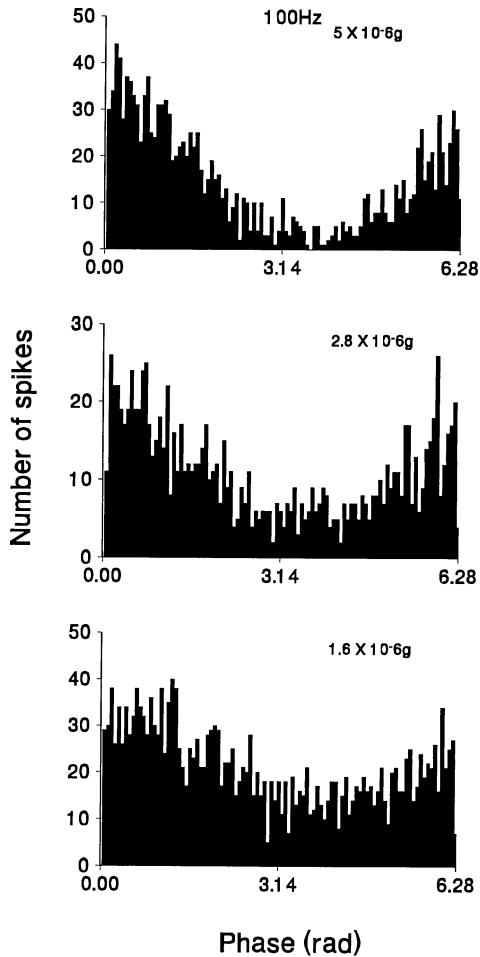
FIG. 5. Phase histograms for a saccular unit of a male white-lipped frog responding to 100 Hz dorsoventral sinusoidal vibration. The peak accelerations of the stimuli are given in gravity units (see Fig. 3 legend).
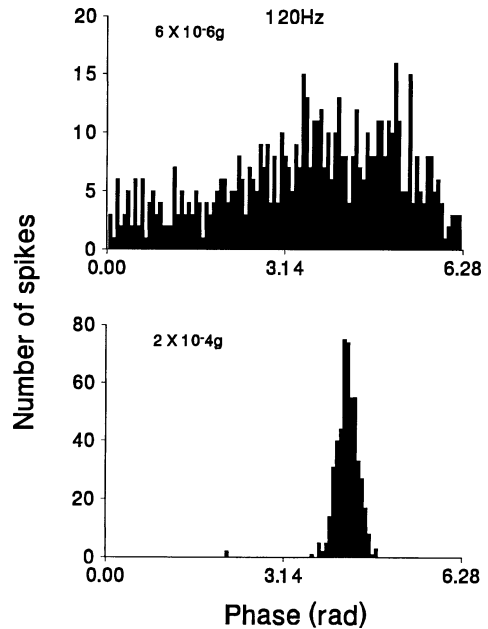

FIG. 6. Phase histogram for a male white-lipped frog saccular unit responding to a 120 Hz sinusoidal vibration. The stimulus amplitude in the lower panel is approximately half of that of the vertical component of a male frog's thump measured at 1.0 m.

the number of occurrences of spike onsets as a function of stimulus phase.

What one clearly sees is a phase-by-phase modulation of the probability of spike production (often called the instantaneous spike rate) by the stimulus sinusoid. The phase is given in radians, with zero phase corresponding to the positive-going zero-crossing of the stimulus (zero phase for a sine function). The stimulus for the bottom panel (approximately 0.000016 m/sec^2) corresponds to peak-to-peak vibratory displacement approximately equal to 8×10^{-11} m. At higher stimulus levels, the smooth sinusoidal modulation of instanta-

neous spike rate gives way to strong phase-locking—all spikes tend to be congregated around a single phase (as seen in Fig. 6 for another *L. albilabris* saccular unit). Figure 7 shows a wide range of peak instantaneous spike rate responses plotted against stimulus amplitude for a saccular unit from *R. catesbeiana* (from Lewis, 1986). The peak responses in the smooth-modulation region at lower stimulus levels (open circles) were computed by discrete Fourier transform applied to the phase histograms. For the strong-phase-locking region at higher stimulus levels, the peak responses (open boxes) were computed by subtracting the mean spike rate in the absence of stimulus (the spontaneous spike rate) from the peak spike rate in the presence of the stimulus. The overall result is thoroughly representative of very large subpopulations (those with substantial spontaneous spike rates) of the saccular units of both frog species. Essentially linear response is seen over a very large dynamic range—three orders of magnitude (60 dB) in the case of Figure 7. The
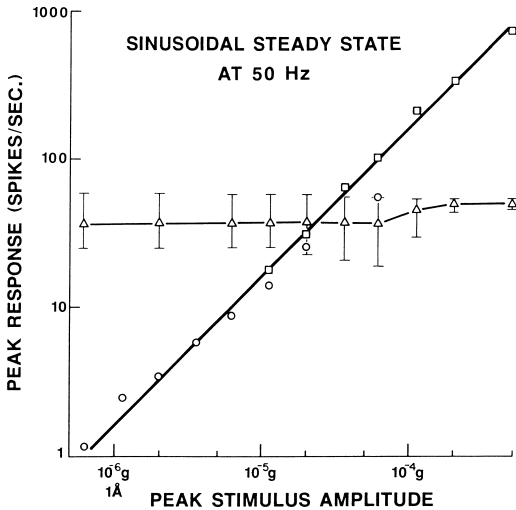
FIG. 7. Stimulus-response plots for a bullfrog saccular unit. The stimulus was a 50-Hz sine wave. The amplitude of the stimulus, given as peak acceleration in gravity units (9.81 m/sec^2), is plotted along the horizontal axis. At 50 Hz, 10^{-6} g corresponds to a peak displacement of approximately 1 Angstrom (10^{-10} m). Triangles show mean spike rate, error bars show positive and negative root-mean-square deviation from that mean. Circles and squares show peak instantaneous spike-rate responses computed by two different methods (see text).

very top of the range shown here corresponds to a peak displacement slightly less than 10^{-7} m, which is near the very bottom of the sensitivity range for most vertebrate somatosensory vibration sensors.

A feature thoroughly representative of all saccular units that we have studied in both frog species is the dependence of the mean spike rate (open triangles in Fig. 7) on stimulus frequency and amplitude. As the stimulus amplitude increased, there was a shift from the mean spontaneous (zero-stimulus) rate (approximately 37 sp/sec in this case) to a spike rate matching the stimulus frequency as the unit became strongly phase locked (producing one spike per stimulus cycle in this case). The error bars in Figure 7 show the root-mean-square deviations of the instantaneous spike rate from its mean value, computed separately for positive and negative excursions. The tightening of the error bars at higher stimulus levels reflects increasingly precise phase locking of the response spikes to the stimulus sinusoid.

The presence of robust linear response

allows us to define a sensor gain for each saccular unit at each sinusoidal frequency. For the unit of Figure 7, for example, at 50 Hz, the sensor gain (peak spike rate divided by peak acceleration) is approximately 2 $\times$ 10^6 spikes/sec per g (or approximately 2 $\times$ 10^5 sp/sec per m/sec^2). With a sequence of sine waves, one could estimate the frequency dependence (tuning) of a unit's sensor gain, one frequency at a time. An alternative method that is far less time consuming is to apply all frequencies at once, in the form of a non-repeating, random (noise) stimulus, then find the cross-correlation between that stimulus and the times of the spikes. This yields a waveform segment that is the mean of the random waveform segments that immediately preceded the spikes (*i.e.,* the mean of what it was in the random stimulus that led to spikes). When that waveform segment is reversed in time, it becomes the first-order Wiener kernel (sometimes called the REVCOR function by hearing researchers), an estimate of the unit's response to an impulsive stimulus (de Boer and de Jongh, 1978; Yu *et al.*, 1991; Eggermont, 1993). The first-order Wiener kernel has proven to be a faithful model of sensory-unit dynamics. With it, one can predict with remarkable precision the temporal pattern of a unit's instantaneous spike rate in response to a novel stimulus waveform of arbitrary spectral content (de Boer and de Jongh, 1978; Wolodkin *et al.*, 1997). The amplitude part of the Fourier transform of the first-order Wiener kernel is a plot of the corresponding frequency dependence of sensor gain. The solid lines in Figure 8 show a first-order Wiener kernel and the amplitude part of its Fourier transform for a saccular unit from *R. catesbeiana*, computed from spike responses to a random dorso-ventral vibration stimulus. Owing to the manner in which they were derived, the kernel itself and its Fourier transform both are noisy. The noise floor, for example, in the upper panel of Figure 8 is approximately -20 dB. A notable feature of the sensor-gain tuning curve of Figure 8, common to the vast majority of saccular units observed so far in both frog species (whether studied with random stimuli or with sine waves,
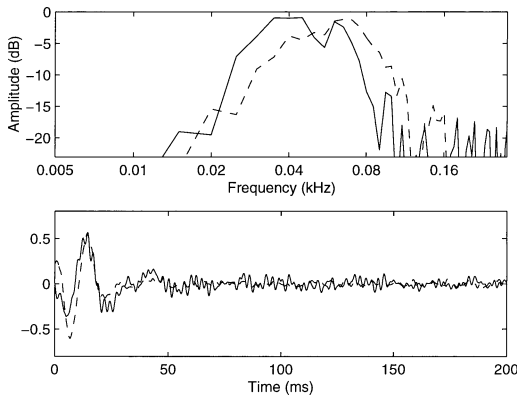
FIG. 8. First-order Wiener kernels (bottom panel) and their Fourier transforms (top panel) for seismic (solid line) and auditory (dashed line) stimuli applied to a bullfrog saccular unit. The waveforms in the lower traces are estimates of the instantaneous spike rate as functions of time following impulsive seismic (solid line) or auditory (dashed line) stimuli. For comparison, the root-mean-square amplitudes of the two waveforms have been scaled to make them approximately equal. Abscissa values in the top panel are estimates of sensor gain (sp/sec per unit stimulus amplitude) plotted log-arithmically (0 dB = 1.0, −20 dB = 0.1). Values in both plots in the top panel were scaled to make the maximum sensor gain approximately 1.0 (0 dB).

one frequency at a time), is its broad band width (more than an octave).

A large proportion of frog saccular units are responsive to airborne sound (auditory stimuli) at moderate levels (see Moffat and Capranica, 1976; Yu *et al.*, 1991). The dashed lines in Figure 8 show the first-order Wiener kernel and the amplitude part of its Fourier transform for the same saccular unit, computed from its spike responses to random sound (noise) delivered through a closed-field system (a tube, sealed around the perimeter of the tympanum). Although there clearly are differences between the two sensor-gain tuning curves of Figure 8, both are broad. Furthermore, the centers of both lie clearly below 100 Hz. This was true for all saccular units that we studied.

As some saccular units respond to auditory stimuli, so do some amphibian-papillar and basilar-papillar units respond to seismic stimuli (Frishkopf and Goldstein, 1963; Yu *et al.*, 1991; ChristensenDalsgaard and Narins, 1993). We found that seismic tuning in *R. catesbeiana* and *L. albilabris* papillar units covered the same frequency range as

auditory tuning (approximately 100 Hz to 1.0 kHz for amphibian-papillar units, above 1.0 kHz for basilar-papillar units). Yu *et al.*, (1991) estimated the seismic sensitivity of such units in *R. catesbeiana* to be approximately one third to one sixth that of typical *R. catesbeiana* saccular units.

REVIEW OF BEHAVIORAL RESULTS WITH CALL PLAYBACKS

In our initial field work in 1983, we found that approximately half of the *L. albilabris* males that we studied produced thumps with their calls. Except during a prolonged dry spell, this has been borne out in our subsequent studies through the years. During the dry spell we found no animals thumping. Immediately after the first rain, thumping animals were abundant once again. We believe that striking of the gular pouch against the ground produces the thumps. This is supported by two pieces of evidence: (1) In one instance, fortuitously, we observed waves being produced on the surface of a thin layer of water in which a thumping frog was sitting. The waves emanated from the gular pouch and were synchronized to the calls. (2) In thumping frogs, the envelopes of the airborne calls tend to show deep amplitude modulations (Fig. 3); those of nonthumping frogs do not (Fig. 4). We presume that these modulations are caused by deformations of the gular pouch as it strikes the ground.

In order to design experiments to test whether or not the seismic waves of the thumps can be used as alternatives to airborne calls in male-male interactions, we decided first to study the interaction involving the airborne call. To do so, we carried out playback experiments with the recorded calls, including that of Figure 4, which was used extensively, played at various repetition rates and at various intensities. In response to playback of recorded calls, a calling frog typically made subtle changes in several parameters (*e.g.,* spectrum, repetition rate, amplitude) of its own call (Lopez *et al.*, 1988). Much more conspicuous, however, was its call timing relative to the playback calls. Figure 9 is representative of our results over a very large number of subjects. The stimulus was the waveform of
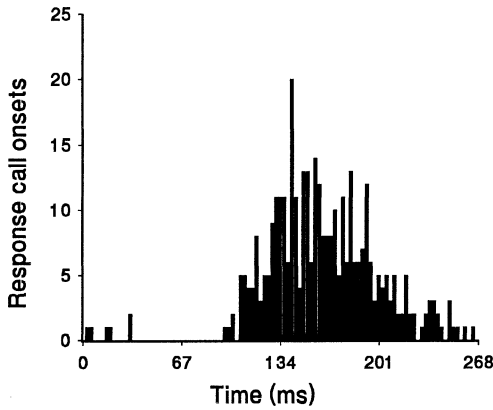
FIG. 9. Histogram showing the call-timing pattern of male white-lipped frogs responding to repetitive playback of the recorded call of Figure 4. Data were accumulated from 25-sec trials with four isolated frogs.
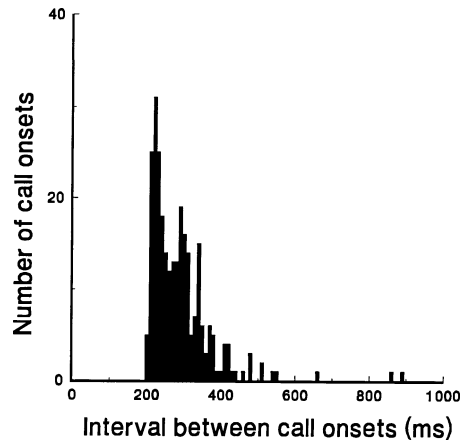

FIG. 10. Distribution of calling intervals for a male white-lipped frog with a 200 msec refractory period. Intervals shorter than approximately 160 msec were never seen.

Figure 4 repeated at a rate of 3.74 sec^{-1} (period of 267 msec), which very close to the natural calling frequency of most of the subjects. Figure 9 shows the sum of the distributions of the onset times, over the 267-msec stimulus period, of the calls of four responding male *L. albilabris* subjects. The value zero on the horizontal axis corresponds to the onset of each stimulus call. The patterns of responses by many subjects to many repetition rates, from 1 to 22 stimulus calls per second, showed that the distribution of Figure 9 resulted largely from three general features of the male *L. albilabris* calling pattern (Moore *et al.*, 1989). (1) There is a tendency to produce calls at a rate of approximately 4 sec^{-1}. (2) There is an absolute refractory period of approximately 160–200 msec (see Fig. 10). (3) There is a strong tendency not to begin a call during an interval of about 30 msec or more, beginning approximately 30 to 40 msec after the onset of the stimulus call. The last feature we labeled the *calling gap,* and we decided to use it as our assay for responsiveness in our male *L. albilabris* subjects. Would those animals produce calling gaps in response to seismic signals?

FURTHER EXPERIMENTS WITH AIRBORNE SOUNDS

To learn more about the calling gap, we employed artificial calls. With those we were able to vary the call duration and spectral composition. We elected to fix the repetition rate of the artificial calls at 4.0 per sec—close to the natural calling rate of our subjects. We elected to use pure tone bursts, with cosine-modulated onsets and offsets to reduce the spectral spread. Among the tone frequencies employed were some selected to be especially excitatory for specific sensors: *e.g.,* 2,200 Hz for the basilar papilla, 650 Hz for the amphibian papilla, and 50 Hz for the saccule. As long as we were dealing with a male frog that was acoustically isolated (not interacting with a nearby calling neighbor), the higher-frequency tone bursts (*i.e.,* 250 Hz and above) elicited conspicuous calling gaps. As the tone-burst duration was increased, the frogs divided into two groups with respect to the pattern of gaps. Figure 11 and 12 show examples of these patterns in response to 2,200-Hz tone bursts with duration ranging from 20 msec to 120 msec. Tone-burst amplitude was 70 dB SPL at the frog's location (where dB SPL refers to decibels of sound pressure level relative to 20×10^{-6} Pa, the decibel measure of sound level familiar to most people). The horizontal lines depict the durations and timing of the stimulus bursts. The consistency of the response patterns can be inferred from Figure 13, which shows histograms for 20-, 40- and 80-msec bursts (2,200-Hz, 70 dB SPL at the frog) summed over seven members
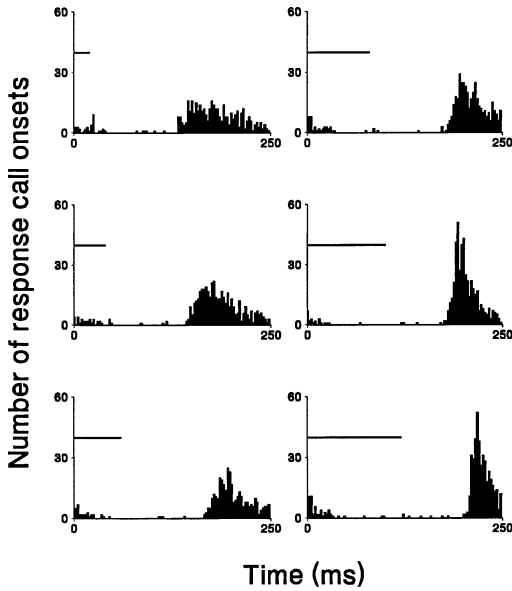
FIG. 11. Calling patterns of a male white-lipped frog in response to repetitive 2,200 Hz tone bursts of various lengths (presented at 4 per sec, 70 dB SPL at the frog). The horizontal line in each histogram indicates the timing and duration of the tone burst.
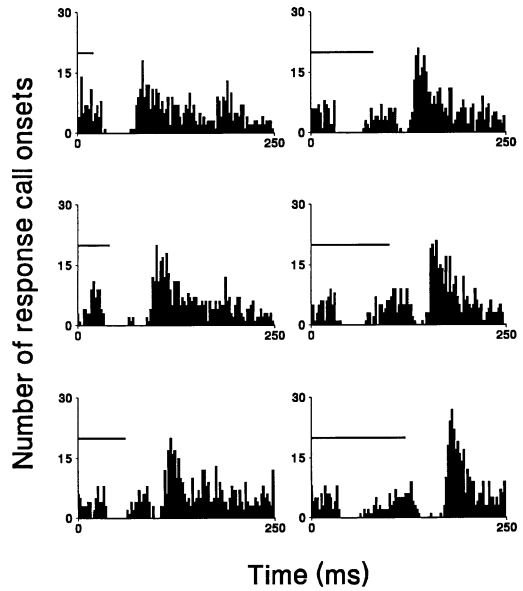

FIG. 12. Calling patterns of another male white-lipped frog. The stimuli were the same as those used for Figure 11.

of one group and five members of the other. In one group, the calling gap commences approximately 40 msec after the onset of the stimulus call and continues until approximately 70 msec after the end of the stimulus call. In the other group, there are two calling gaps: one commencing approximately 40 msec after the onset of the stimulus call, the other commencing approximately 40 msec after the end of the stimulus call. The second gap is somewhat narrower than the first.

Because the power of the *L. albilabris* thump is highly concentrated in the frequency range of the saccule (well below 100 Hz), extending very little into the lowest-frequency parts of the amphibian papilla, we were especially interested in the ability of 50-Hz tone bursts to elicit calling gaps. Figure 14 shows a result from one of the subjects from the left-hand column of Figure 13. To avoid excessive spectral spread, we selected 60 msec (three periods) as the minimum duration for our 50-Hz bursts. Both tone bursts used for Figure 14 had 70 dB SPL amplitudes at the location of the frog. Of the eleven subjects for which
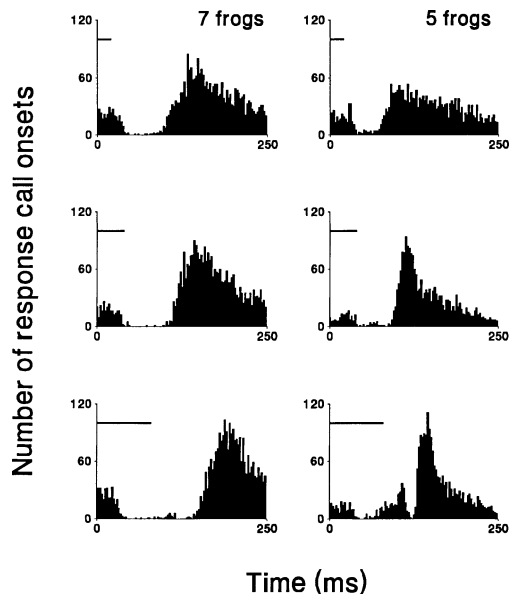

FIG. 13. Cumulative histograms taken over the two groups of white-lipped frogs (see text), showing the calling patterns for a subset of the stimuli used in Figures 11 and 12.
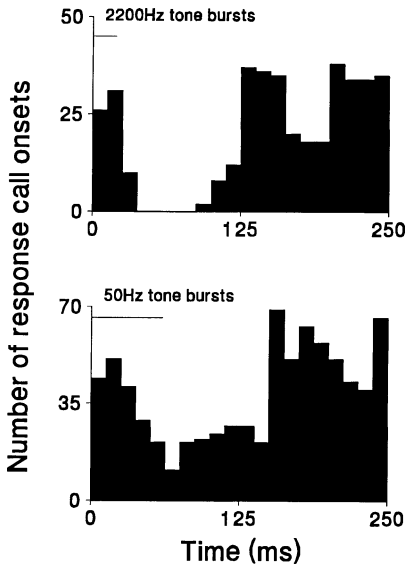
FIG. 14. Calling patterns of a male white-lipped frog in response to tone bursts at 50 Hz and 2,200 Hz presented at 4 per sec, with amplitudes of 70 dB SPL measured at the frog.
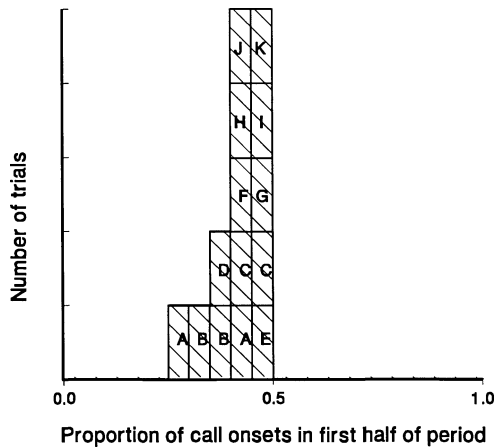

FIG. 15. Distribution of responses of 14 male white-lipped frogs to 50-Hz tone bursts. Frogs A, B and C each were subjected to two trials. In each trial, calls were accumulated over a period of 120 sec, yielding approximately 450 response calls.

this experiment was carried out (eleven of the twelve frogs represented in Fig. 13), only two responded to the 50-Hz tone burst with calling gaps as conspicuous as this. Even with those two subjects the depth of the gap was inconsistent from one trial to another. As a crude but simple measure of the tendency to produce this gap in response to the 50-Hz tone burst (60 msec, 70 dB SPL), we took the ratio of call response call onsets in the first half of the 250-msec stimulus period to the total number of call onests. Figure 15 shows a histogram of the results taken over all fourteen trials that we carried out on the eleven subjects (labeled A through K). Each trial was carried out for 120 sec and accumulated approximately 450 response calls. Although the stimulus clearly was effective, its actual effects were inconsistent within and between subjects.

EXPERIMENTS WITH SEISMIC THUMPS

Figure 16 shows the device (thumper) we currently use to produce thumps. We found that seismic waveforms (observed with geophones) produced by impulsive mechanical stimuli (including those produced

by the thumper), are largely determined by the physical properties of the ground and largely independent of the stimulus source. Therefore we were able to match very well the amplitude, shape, and spectrum of the seismic thump waveforms produced by the frogs. Our experiments were carried out at night (between 1800 and 0200 hr), in the Luquillo Mountains of eastern Puerto Rico. Figure 17 depicts our general setup in the field. Not all of the elements of Figure 17 were actually deployed in all of the specific
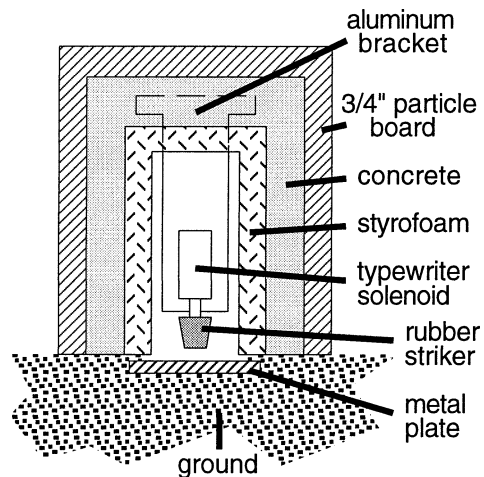

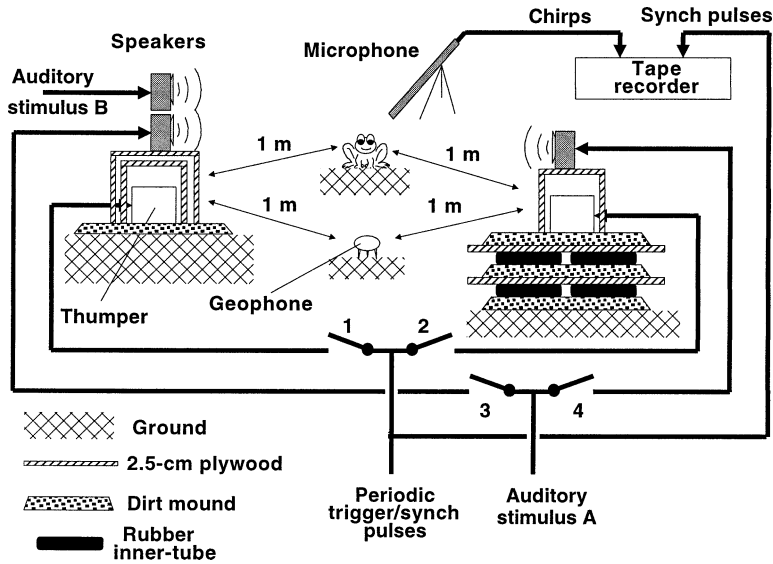FIG. 16. Configuration of the thumper used in our most recent experiments.

FIG. 17.   Generalized diagram of the field setup. Two tape players and one tape recorder were used. Prerecorded stimulus patterns, comprising periodic trigger/synch pulses and auditory stimuli (*e.g.,* thump sounds, recorded calls, artificial calls, noise bursts) synchronized to those pulses were presented from one tape player. Prerecorded, continuous, broad band auditory noise was presented from the second tape player. The response calls (chirps) from the frog and the periodic trigger/synch pulses from the first tape player were recorded simultaneously on separate channels of the tape recorder.

setups. Because it is impossible to generate impulsive seismic waves without concomitant impulsive airborne sounds, we had to take special care to rule out the possibility that our *L. albilabris* subjects were responding to the airborne components rather than the seismic components of our stimuli. As one can infer from Figure 17, we attempted to do this in several ways. In our earlier experiments, we observed the minimum amplitude of airborne sound (noise or tone) bursts that would evoke gap production. Then, with the thumper (on the ground) covered by three wooden boxes (one more than shown on the left in Fig. 17), we adjusted the thump amplitude until the peak thumper sound level was between one third and one tenth of that observed minimum. In our later experiments, we attempted to determine the responsiveness of each subject to simulated thumper sounds either by playing recordings of those sounds or by generating approximations of those sounds with a thumper mounted on a vibration isolation platform (shown on the right in Fig. 17). Then we added continuous airborne noise (20 Hz–16 kHz) to mask the thumper

sounds. Effective levels for masking were determined by applying the noise together with simulated thumper sounds. In all of these experiments, we presented periodic stimuli (thumps, simulated thumper sounds, and calls) at a rate of 4 sec^{-1}.

In both our earlier and later experiments, inconsistency within and between subjects was comparable to that with auditory stimulation of the saccule (bottom panel of Fig. 14, Fig. 15). In both cases approximately 15–20% of subjects produced conspicuous calling gaps in response to seismic thumps whose concomitant sound was masked or reduced to subthreshold levels. The masking-noise experiments, for example, were carried out on fifteen subjects. Among those fifteen animals, only three showed conspicuous gap production in response to the combination of thumps and masking noise. Figures 18 and 19 show results from two of those three animals.

Figure 18 shows the results for an isolated subject on the grounds of the El Verde Field Research Station. The top panel shows the distribution of call onsets in response to recorded thumper sounds, played
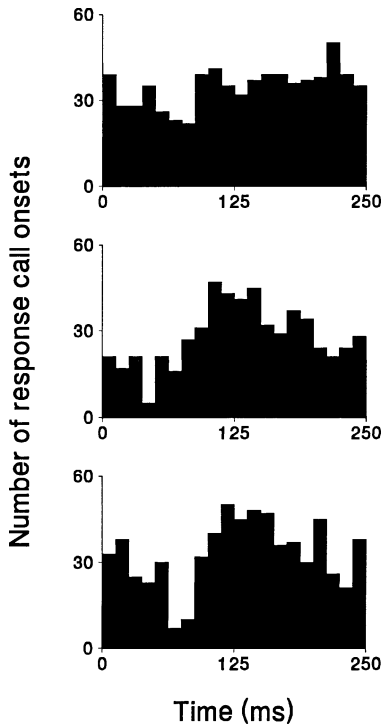
FIG. 18. Call patterns of a male white-lipped frog in response to recorded thumper sounds (top panel), to thumps with accompanying sounds (middle panel), and to thumps with accompanying sounds plus masking noise (bottom panel).
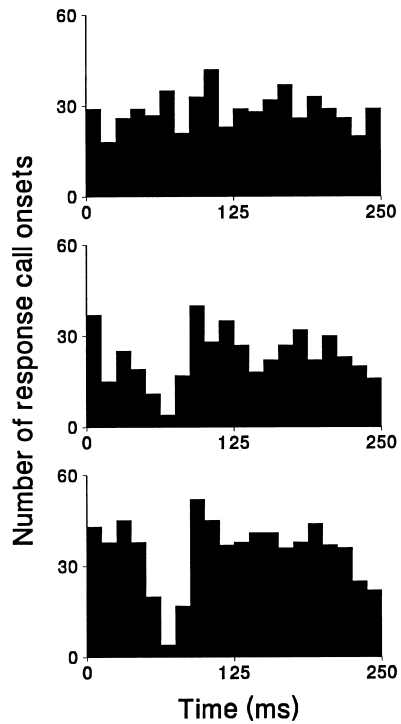

FIG. 19. Call patterns of another male white-lipped frog in response to thumps (top panel), to thumps plus masking noise (middle panel), and to thumps plus masking noise at a slightly higher level (bottom panel).

with peak amplitude of 54 dB SPL (set to match the amplitude of the sounds from the thumper on the ground), with no masking noise. The middle panel shows the distribution in response to periodic thumps from the thumper on the ground, with no masking noise. The bottom panel shows the distribution in response to the thumper in the presence of masking noise (20 Hz–16 kHz), presented at 72 dB SPL. The peak amplitude of the sound from the thumper was 54 dB SPL. The power of the thumper sound was distributed largely between 100 Hz and 3 kHz. In response to the thump sounds alone there appears to have been a subtle tendency not to initiate a call in the first half of the stimulus period. In response to the unmasked thumper on the ground, this tendency became distinct, and is followed by the usual rebound of calling activity. When masking noise was added (bottom panel), a conspicuous gap appeared (between 62.5 and 87.5 msec following the onset of the stimulus).

Figure 19 shows the results for a subject in a small patch of wet meadow. The frog was within a few meters of other calling males. The top panel shows the distribution of call onsets in response to periodic thumps from the thumper on the ground, with no masking noise. The middle panel shows the distribution in response to the same thumps, presented with noise (20 Hz–16 kHz) at 77 dB SPL. The bottom panel shows the distribution in response to the same thumps, presented with the same noise at 80 dB SPL. In that panel, a conspicuous gap is evident between 50 and 87.5 msec following the stimulus onset. The peak thumper sound was 50 dB SPL, again with its power distributed largely between 100 Hz and 3 kHz. The results in these three panels suggest that the subject was focusing on the sounds of calling neighbors and ignoring the thumper—until

the presence of the high level of noise effectively blocked the airborne channel. With both of these subjects (that of Fig. 18 and that of Fig. 19), the peak vertical acceleration amplitudes of the seismic thumps were in the low 10^{-4} g range, matching the typical amplitude at 1 m distance of the thump from a calling frog. From Figures 6 and 7, it can be seen that these amplitudes are sufficient to produce very strong, highly synchronized responses among the frog's saccular units.

DISCUSSION

The three elements of circumstantial evidence presented in the synopsis now can be restated as follows: When the ground is damp, approximately half the calling male white-lipped frogs assume a posture that allows the gular pouch to strike the ground during each call. Each of the resulting seismic signals has a waveform shape and spectrum that is typical of those from impulsive stimuli in general (such as predator footfalls) applied to the same substrate. The white-lipped frog saccule is exceedingly sensitive and is well-tuned to impulsive seismic signals in that substrate.

Thus one can argue that the seismic signal produced by the male white-lipped frog is an accidental byproduct of calling posture. On the other hand, calling posture might be an adjustable parameter of communication for the calling male. We have not addressed that possibility in our experiments. Whether or not the seismic signal is accidental, the frog's saccule clearly is well-suited for sensing it. The question we have addressed in our behavioral experiments so far is whether or not there are circumstances under which other male frogs would adjust their own calling parameters in response to it. Figures 18 and 19 provide definitive evidence that there are. In Figure 18, the combination of seismic stimulus and auditory stimulus (thump sound) produced a conspicuous adjustment by an isolated subject (center panel), where the same auditory stimulus by itself did not (upper panel). When the auditory stimulus was thoroughly masked by continuous airborne noise, so that only the seismic stimulus remained, there also was a conspicuous ad-

justment (bottom panel). The same thing is true for the bottom panel of Figure 19; when auditory stimuli (from other frogs in this case) were masked by continuous airborne noise, the subject responded conspicuously to the seismic stimulus. Because the saccule itself is sensitive to airborne sound, one expects that there would be a level of acoustic noise in the air sufficient to mask the saccular response to any given seismic stimulus. Figure 19 suggests, however, that there is a range of such noise levels sufficiently high to mask airborne calls yet sufficiently low to allow the saccule to detect thumps.

REFERENCES

Ashcroft, D. W. and C. S. Hallpike. 1934. On the function of the saccule. J. Lar. 49:450–458.

Autrum, H. and W. Schneider. 1948. Vergleichende Untersuchungen uber den Erschutterungssinn der Insekten. Z. Vergl. Physiol. 31:77–88.

Cazin, L. and J. Lannou. 1975. Response du saccule a la stimulation vibratoire directe de la macule, chez l grenouille. C. R. Soc. Biol. 169:1067–1071.

ChristensenDalsgaard, J. and P. M. Narins. 1993. Sound and vibration sensitivity of VIIIth nerve fibers in the frogs *Leptodactylus albilabris* and *Rana pipiens pipiens*. J. Comp. Physiol. A 172: 653–662.

de Boer, E. and H. R. de Jongh. 1978. On cochlear encoding: Potentialities and limitations of the reverse correlation technique. J. Acoust. Soc. Am. 63:115–135.

Dunn, R. F. 1978. Nerve fibers of the eighth nerve and their distributions to the sensory nerves of the in-

ner ear in the bullfrog. J. Comp. Neurol. 182:621–636.

Eggermont, J. J. 1993. Wiener and Volterra analysis applied to the auditory system. Hear. Res. 66:177–201.

Frantti, G. E., D. E. Willis, and J. T. Wilson. 1962. The spectrum of seismic noise. Bull. Seism. Soc. Am. 52:113–121.

Frishkopf, L. S. and M. H. Goldstein, Jr. 1963. Responses to acoustic stimuli from single units in the eighth nerve of the bullfrog. J. Acoust. Soc. Am. 35:1219–1228.

Gualttierotti, T. 1968. Analysis of single vestibular responses. *In* A. S. Iberall and J. B. Beswick (eds.), *Technical and biological problems of control,* pp. 318–331. Federation of Automatic Control, Pittsburgh.

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

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

Lewis, E. R. 1972. Structural-functional correlates in inner ear receptors. *In* C. J. Arceneaux (ed.), *Proceedings of the 30th annual meeting,* Vol. 6, pp. 64–65. Electron Microscopy Society of America, Los Angeles.

Lewis, E. R. 1986. Adaptation, suppression and tuning in amphibian acoustical fibers. *In* B. C. J. Moore and R. D. Patterson (eds.), *Auditory frequency selectivity,* pp. 129–136. Plenum, New York.

Lewis, E. R. and C. W. Li. 1973. Evidence concerning the morphogenesis of saccular receptors in the bullfrog (*Rana catesbeiana*). J. Morph. 139:351–361.

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

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

Lewis, E. R. and P. M. Narins. 1981. Seismic sensitivity in eighth nerve afferent fibers of the white-lipped frog. Soc. Neurosci. Abs. 7:148.

Lewis, E. R. and P. M. Narins. 1985. Do frogs communicate with seismic signals? Science 227:187–189.

Lewis, E. R. and P. M. Narins. 1998. The acoustic periphery of amphibians: Anatomy and physiology. *In* R. R. Fay and A. N. Popper (eds.), *Comparative hearing: Fish and amphibians,* pp. 101–154. Springer-Verlag, New York.

Lombard, R. E. and I. R. Straughan. 1974. Functional aspects of the anuran middle ear structure. J. Exp. Biol. 61:71–93.

Lopez, P. T., P. M. Narins, E. R. Lewis, and S. W. Moore. 1988. Acoustically-induced call modification in the white-lipped frog *Leptodactylus albilabris*. Anim. Behav. 36:1295–1308.

Moffat, A. J. M. and R. R. Capranica. 1976. Auditory sensitivity of the saccule in the American toad. J. Comp. Physiol. 105:1–8.

Moore, S. W., E. R. Lewis, P. M. Narins, and P. T. Lopez. 1989. The call-timing algorithm of the white-lipped frog, *Leptodactylus albilabris*. J. Comp. Physiol. A 164:309–319.

Narins, P. M., G. Ehret, and J. Tautz. 1988. Accessory pathway for sound transfer in a neotropical frog. Proc. Natn. Acad. Sci. U.S.A. 85:1508–1512.

Narins, P. M. and E. R. Lewis. 1984. The vertebrate ear as an exquisite seismic sensor. J. Acoust. Soc. Am. 76:1384–1387.

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

Wolodkin, G., W. M. Yamada, E. R. Lewis, and K. R. Henry. 1997. Spike rate model for auditory nerve fibers. *In* E. R. Lewis, G. R. Long, R. F. Lyon, P. M. Narins, C. R. Steele, and E. Hecht-Poinar (eds.), *Diversity in auditory mechanics,* pp. 104–110. World Scientific, Singapore.

Yu, X. L., E. R. Lewis, and D. Feld. 1991. Seismic and auditory tuning curves from bullfrog saccular and amphibian papillar axons. J. Comp. Physiol. 169:241–248.