Auditory and Vibratory Responses in the Midbrains of Snakes

Abstract. *Airborne sound and substrate vibration each elicit electrical responses below the surface of the tectum in species of three families of snakes. Tones of 50 to 1000 hertz evoke responses independently of substrate vibration. Sensitivity to locally applied sound is present over much of the body surface. This sensitivity is attributed to the auditory nerve, because it is not altered by spinal section but is eliminated by destruction of the inner ear.*

Snakes lack even the rudiments of an external ear and have been considered insensitive to airborne sound (*1*). The columella's articulation with the quadrate bone and consequent indirect connection to the mandible have been considered as adaptations for reception of vibratory stimuli from the substrate (*1*).

This anatomical evidence has been supported by the absence of behavioral responses to auditory stimuli and by numerous anecdotal accounts of responses to vibration (*2*). Wever and Vernon (*3*) showed that snakes do exhibit cochlear microphonics, thus demonstrating a peripheral auditory response. We investigated neural responses to sound and possible neural bases of the reported vibration sensitivity (*4*). Electrophysiological evidence suggests the presence of a sensory modality for auditory and substrate vibratory stimuli. We advance the hypothesis that sound incident on the snake's body is transmitted by a mechanical path to the ear where it is transduced into a neural response.

Snakes representing the families Boidae (*Boa constrictor, Eunectes murinus*, the anaconda, and *Corallus cooki*, a tree boa), Colubridae (*Pituophis melanoleucas*, the gopher snake), and Crotalidae (rattlesnakes *Crotalus viridis* and *C. cerastes*) were examined. The specimens were anesthetized with pentobarbital sodium (15 to 30 mg per kilogram of body weight, a dosage not greatly reducing the midbrain responses). Room temperature was kept within the snakes' normal temperature range, between 25° and 29°C. The tectum was exposed, and a fine tungsten wire or metal-filled glass-pipette electrode was inserted to a depth of 1 to 3 mm. For some preparations the electrode was fixed in a favorable location with dental cement.

Responses were amplified and recorded in a conventional manner. Responses were often small compared to ongoing slow-wave activity; ten or more such responses were therefore averaged by digital techniques. The sound stimuli were tone bursts of constant frequency (*5*) delivered by a headphone or a loudspeaker. Sound pressure level (SPL), intensity in decibels relative to 0.0002 dyne/cm^2 (r.m.s.), was measured in a mock experimental setup with a calibrated microphone.

Figure 1A (top) (*1*) shows a single slow-wave response in a preparation exceptionally free from spontaneous slow activity. The long latency (about 40 msec) could only be slightly shortened by use of higher frequencies and intensities, faster rise times, or click stimuli. The amplitude of the response depended on the position of the electrode; vertical displacements of 200 μ eliminated the responses in some preparations. The spikes in Fig. 1A (bottom) (*2*) were recorded at the same site as was a slow-wave response (filtered out in this record). Spikes have not been observed to be phase locked to the sound pressure waves in any units at this level in the auditory pathway, regardless of the frequency of stimulation.

Figure 1B shows representative averaged responses recorded from snakes of three families to sounds of identical frequencies and rise-fall times. Similar records were obtained from three other species. The shape of the averaged response does not depend greatly on the side of the snake on which the sound is delivered (Fig. 2A). Both shape and latency, however, depend on the recording site, the temperature, the electrode size, and the intensity of the stimulus. Inverted responses, responses with little positive wave or with a large preceding positive wave, were recorded.

All of the snakes tested have been maximally sensitive to sound in the range 75 to 200 hz. Sound pressures in excess of 100 db (SPL) were required to elicit responses to tones above 1000 hz. At 50 to 60 hz, the lowest frequency available with our loudspeakers, the sensitivity remains high. Experiments on a table with good vibration isolation in a sound-proofed room show responses to tones between 100 and 200 hz with SPL of less than 40 db (number averaged = 50). Thus, averaged slow potentials indicate that, at the very low frequencies, snakes have an auditory sensitivity approaching that of some primates. In contrast, snakes seem severely limited at frequencies greater than 1000 hz. These facts are in accord with the observations of Wever and Vernon (*3*).

Vibration stimuli could be generated either by tapping the experimental table or by laying the snake across a 10-cm platform resting on the cone of a small loudspeaker. This platform could then be driven by the same waveforms used to produce sound. Responses to vibrations occurred whenever sound responses were obtained, and had shapes and latencies similar to those for sound. The range of frequencies of vibration effective in causing responses was approximately the same as the range for sound.

Both sound and vibration responses have distributed peripheral origins. The sensitivity pattern for sound was carefully measured by a sound field which fell in intensity by 3 to 4 db per centimeter of displacement from the point of focal stimulation. Figure 2A shows the result for one snake (*C. viridis*; specimen length was 30 cm). The details of the pattern were not the same for the other two snakes (of different species) for which measurements were made. In all cases, however, the sensitivity extended past the middle of the snake. The distributed origin of sound and vibration responses suggests two explanations. There may be a local response originating at the site of stimulation and being transmitted to the brain over segmental nerves, or there may be a pathway, external or internal to the snake, by which vibrations coming from air or substrate reach a sensor at the head.

Anatomical manipulations were used to distinguish among these possibilities. The use of an implanted electrode assured that recordings made before and after such manipulations would be nearly equivalent as to electrode location. Snakes with implanted electrodes could be picked up, allowed to recover from barbiturate, reanesthetized, and dissected in various ways without any great changes in response to sound. A tone of 140 hz, with an SPL of 80 db, was delivered 30 cm posterior to the head of one such snake (*Pituophis*). The averaged response was obtained. The spinal cord was then severed with bone clippers, and the snake was maintained under artificial respiration. The

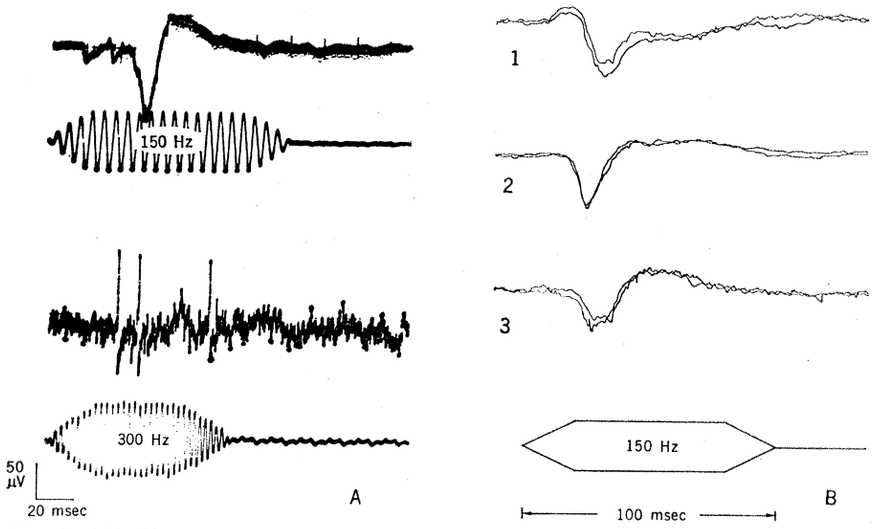

Fig. 1. (A) Slow response (top) and spike response (bottom) to sound-tone bursts whose envelopes appear as the second trace in each record. (B) Averaged responses ($n = 20$) to sound-tone burst, 70 db SPL, whose envelope is shown above time calibration; (record 1) *Boa constrictor*; (record 2) *Crotalus cerastes*; (record 3) *Pituophis melanoleucas*. Two averaged responses superimposed for evaluation of noise. Positive upward.

response to sound was undiminished, therefore spinal nerves were not essential. After bilateral destruction of the inner ear, however, no response was obtained for sounds of 100 db SPL. The brain was still functional to the extent demonstrated by subsequently recorded responses to flashes of light. The possibility remains that the electrode was displaced by the ear destruction, but this is regarded as unlikely since the implant had been extremely stable in previous manipulations. In an experiment with one *Crotalus viridis* both sound and vibration responses were found for mid-snake stimulation after the spinal cord was severed, but ear destruction was not attempted. These experiments suggest that spinal nerves do not mediate the sound or vibration responses.

Sound from the earphone causes small sympathetic vibrations in the snake's substrate. These might be transmitted as substrate vibrations to the region of the snake's head and might there be perceived as vibrations. Figure 2B contraindicates this possibility. In record 1, a 100 hz 70 db SPL tone burst was delivered locally to a boa's head. In records 2 and 3, an electromagnetic transducer was used to produce a sinusoidal displacement of the substrate (a lead brick) at 100 hz. The second line in each record shows the output of a semiconductor strain gage arranged as an accelerometer and attached to the brick. The strain gage had a mechanical resonance at 200 hz, which introduced a second harmonic in the measurement persisting for 150 msec. The sound response was present when no vibration was detected by the accelerometer (record 1). The accelerometer was sufficiently sensitive to detect vibrations of the brick which produced no response (record 2). Sufficiently large vibrations did evoke responses (record 3). Therefore the sound itself gave rise to the response in record 1. If a brain response is accepted as indicative of hearing, these snakes can hear airborne sound.

These results indicate that airborne sound alone or substrate vibration alone can lead to a midbrain response. We have not conclusively ascertained the degree of independence of the peripheral or central pathways. Generally, the responses to tone bursts delivered with the vibration transducer look similar in shape and latency to responses to airborne tone bursts. (In Fig. 2B, records 1 and 3 show an exception which might be accounted for in terms of relative intensity of the stimuli.) The populations of neurons responding to sound and vibrations probably overlap to a large extent.

The sensitivity of snakes to vibration is remarkably great and might easily provide a basis on which to explain the anecdotes of vibration sensitivity. Our best hypothesis is that sound or vibration incident on the snake's body is transmitted to the inner ear by a mechanical path. The ear transduces the tissue-borne sound into a neural response which enters the brain over the auditory nerve.

Berman and Regal (*6*) advanced the hypothesis that, in snakes, selective pressures related to feeding mechanisms have caused changes of ear structure without necessarily disturbing its function. Our data support this thesis but suggest that the auditory pathway re-

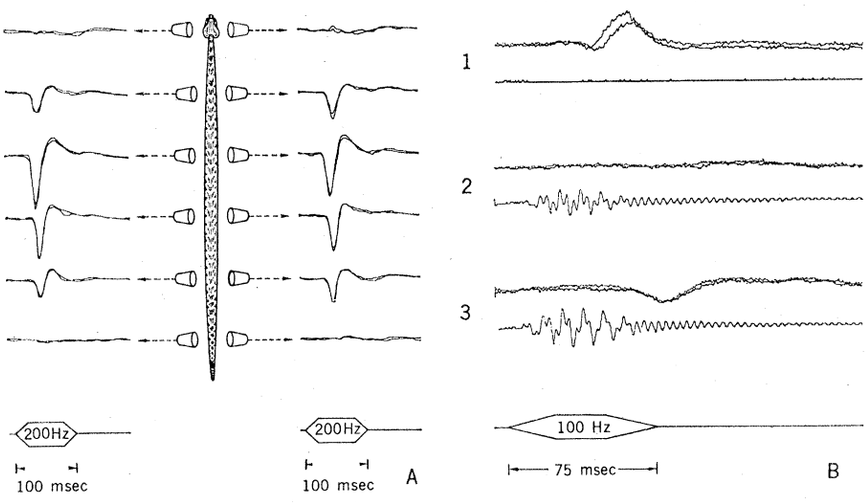

Fig. 2. (A) Averaged responses ($n = 20$) of *Crotalus viridis* to localized sound. Position of sound source indicated for each trace by horn and arrow. Intensity opposite horn, 70 db SPL. (B) Averaged responses ($n = 64$) of *Pituophis melanoleucas* to sound (record 1) and to vibration (records 2 and 3). Stimulus envelope, indicated above time calibration, was same for sound and vibration. Second trace in each pair is the output of accelerometer attached to platform holding snake. Positive upward in all records.

sponds to vibration as well as sound. Thus, if snakes cannot "hear" it is not for want of neural information about sounds.

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References and Notes

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2. F. B. Manning, *J. Comp. Psychol.* **3**, 241 (1923).
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4. This research was reported at the 24th International Congress of Physiological Sciences, 29 August 1968. The abstract [P. H. Hartline and H. W. Campbell, *Proc. Int. Union Physiol. Sci.* **7**, 183 (1968)] was modified in the oral presentation with respect to the independence of neural pathways subserving vibration and sound modalities.
5. The tone-burst modulator was designed and built by R. H. Hamstra, Jr. Frequency range: d-c to 200 khz, amplitude on-off ratio greater than 90 db up to 20 khz and decreasing to about 70 db at 200 khz. No on or off transients are measurable.
6. D. S. Berman and P. J. Regal, *Evolution* **21**, 641 (1967).
7. We thank Dr. T. H. Bullock for advice and many associates for criticism of the manuscript. Part of the research was carried out on the R.V. *Alpha Helix* 1967 Amazon Expedition of the Scripps Institution of Oceanography. Supported by grants to Dr. T. H. Bullock from NSF, NIH, USAF Office of Scientific Research, and ONR, and by postdoctoral fellowships to P.H.H. and H.W.C. from NSF and NIH.

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Bullfrog (Rana catesbeiana) Ventilation: How Does the Frog Breathe?

Abstract. *Two short-term cyclic events, buccal oscillations and ventilatory cycles, occur in the bullfrog's respiration. During ventilation the frog fills the buccal cavity with air, then blows the pulmonary contents through the buccal cavity, and finally closes the nostrils while pumping the buccal contents into the lungs. The pulmonary efflux streams by the buccal contents with minimal mixing, and relatively pure air is pumped into the lungs. Buccal oscillations serve mainly to flush out the buccal cavity between ventilatory cycles.*

The mechanism of lung filling in frogs differs from that of higher vertebrates in that it consists of a compression rather than a suction mechanism (*1*), but considerable disagreement exists about its details. Myographic analysis of respiration in the bullfrog *Rana catesbeiana* (*2*) reveals two kinds of cycles. The first, buccal oscillation cycles, proceed with the nostrils open and the glottis closed; air is alternately forced into and out of the buccal cavity by contractions of the buccal floor. During the less frequent ventilatory cycles, the glottis opens after the buccal floor has been depressed. Pulmonary gas, at a pressure which is always above atmospheric pressure, then enters the buccal space. This inflow induces a rise in buccal pressure and a simultaneous outflow of gas through the open nostrils. The nostrils then close, and a sudden contraction of all of the subbuccal muscles sharply increases the buccal pressure, driving some of the gas from the buccal cavity into the lung. The glottis closes near the peak of the buccal pressure, leaving the lung at a pressure substantially above atmospheric pressure. The buccal floor finally depresses while the nostrils open so that ambient air flows in to refill the buccal space.

Relatively little is known about volumetric relationships, particularly since these vary drastically, even between successive cycles (*2*). The lung of a 300-g frog ordinarily seems to hold 30 to 60 ml of gas, whereas actual tidal volumes are less than the 6- to 15-ml volume of the buccal cavity. Observed tidal volumes were low and varied by a factor of 10 as a function of behavioral parameters (*3*). This suggests that statements (*1*) that pulmonary gas is mixed with the contents of the buccal cavity and that the mixture is subsequently rebreathed into the lungs might be correct, so that the process seems most inefficient.

Yet at least three factors suggest that those buccal gases ultimately shunted into the lungs have not suffered random admixture of pulmonary efflux. (i) Most of the buccal volume lies in a posterior chamber, ventral to the opening of the larynx and surrounded by the petrohyoid muscles. (ii) The glottis is vertical and faces directly forward. Gases escaping from it under pressure might tend to pass in a stream through the distended buccal cavity and to impinge on the internal nares. (iii) The petrohyoid muscles fire during ventilation but not during oscillation cycles. Gas escaping from the lungs may thus pass directly forward, bypassing the posterior portion of the buccal cavity, with its contents remaining relatively unmixed. The unmixed gas would then be driven into the lung during the inflow phase of the ventilation cycle, and the bypassing mechanism would reduce the pumping work required because almost none of the inhaled gas is derived from the previous expiration.

We monitored gas leaving the nostrils of adult bullfrogs (*Rana catesbeiana*) with a respiratory mass spectrometer (*4*). The gas-sampling probe was set in the center of a tube (5 to 15 mm long with an internal diameter of 2 mm) placed opposite the nostrils by a mask (Fig. 1). The mask, cast individually from a quick-set rubber compound, covered the surface of the upper jaw, and curved slightly around the upper lip. Its lateral and dorsal wings were sutured, respectively, to the sides of the face below the eyes, and to the skin of the back. Because the posterior edges were thinned, there was minimal interference with the frog's eyes and ears. The internal surface of the mask was cut back to allow full freedom of nostril movement. Gas concentrations in the buccal efflux and buccal pressure were determined simultaneously (Fig. 2) on unrestrained and unanesthetized animals placed beneath an inverted bar-mesh basket.

In the first experiment the frog was permitted to breathe air. In the second experiment frog and basket were placed into a 4-liter desiccator jar, the contents of which could be rapidly flushed with a mixture of 80 percent argon and 20 percent oxygen or with air. Replacement (99 percent) was achieved in less than 15 seconds for a flow rate of 3 to 4 liter/sec.

In room air oxygen contents of the nasal efflux directly reflected the changes in buccal pressure. The nasal efflux for each ventilatory cycle corresponds to a minimum oxygen value (approximately 1 to 2 percent below ambient). Estimates based on the efflux during the first oscillatory cycle after a ventilation suggest that the nasal efflux contains between 25 and 50 percent of