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## **Auditory Sensitivity of the Northern Pacific Rattlesnake, *Crotalus viridis oreganus*: Do Behavioral Responses Conform to Physiological Performance?**

### **Abstract**

Previous physiological studies demonstrated that snakes, including rattlesnakes of the genus *Crotalus*, are capable of responding to airborne sounds, but only over a relatively narrow range of frequencies. To assess the accuracy of these frequency limitations, this study presented Northern Pacific rattlesnakes (*C. viridis oreganus*) with a range of synthesized airborne tones. Significant levels of positive responses, as evinced by a suite of behaviors, were observed outside of the physiologically-determined frequency ranges. The results of the present study suggest that hearing in rattlesnakes, and perhaps other snakes, is more biologically relevant than previously appreciated.

### **Introduction**

Contrary to the persistent popular misconception that they are deaf, a variety of experimental evidence has clearly demonstrated that snakes, including rattlesnakes, can perceive and respond to airborne and groundborne vibrations. Hartline (1969) used intracellular recordings of auditory mid-brain neurons to document the range of auditory sensitivity in the prairie rattlesnake (*Crotalus viridis*). Subsequently, Wever (1978) used a different technique, the measurement of cochlear potentials, to also determine the auditory sensitivity range of *C. viridis*. Though the recording techniques differed between these two studies, much of their methodology was similar: the rattlesnakes used were of a similar size, all recordings were taken from anesthetized specimens, and the rattlesnakes were presented with only pure tones which lacked temporal patterning, amplitude modulation, or frequency modulation (Hartline, 1969; Wever, 1978). The auditory sensitivity curves produced by these two studies are in general agreement regarding the peak sensitivity, but the curve produced by Wever (1978) is more obtuse resulting in a broader frequency

range (Fig. 1). This discrepancy may be due to Wever's reliance on measurements of cochlear potentials; other researchers (e.g., Manley, 1990) have argued that cochlear potentials can lead to inaccurate auditory sensitivities.

Young and Aguiar (2002) demonstrated that the western diamondback rattlesnake (*Crotalus atrox*) would behaviorally respond to airborne vibrational stimuli. Their study used a special chamber to eliminate groundborne vibration, and documented four behavioral responses from the rattlesnake when presented with airborne sounds: changes in tongue-flick rate, rapid lateral movement of the head (a head jerk), maintained cessation of movement (a freeze), and rattling. Young and Aguiar (2002) presented the rattlesnakes with synthesized tones that included temporal patterning, amplitude modulation, and frequency modulation. Although other claims have been made on anecdotal evidence (see Young, 2003 for a review), the study of Young and Aguiar (2002) offers the only experimental evidence that any rattlesnake can respond behaviorally, that is to say, can hear, an airborne sound.

The present study was undertaken in an attempt to more fully integrate, and expand upon, these earlier studies. More specifically, this study was intended to: 1) document whether or not species of rattlesnake, beside *Crotalus atrox*, will respond to airborne sounds, 2) examine whether or not behavioral responses could be evoked by pure tones

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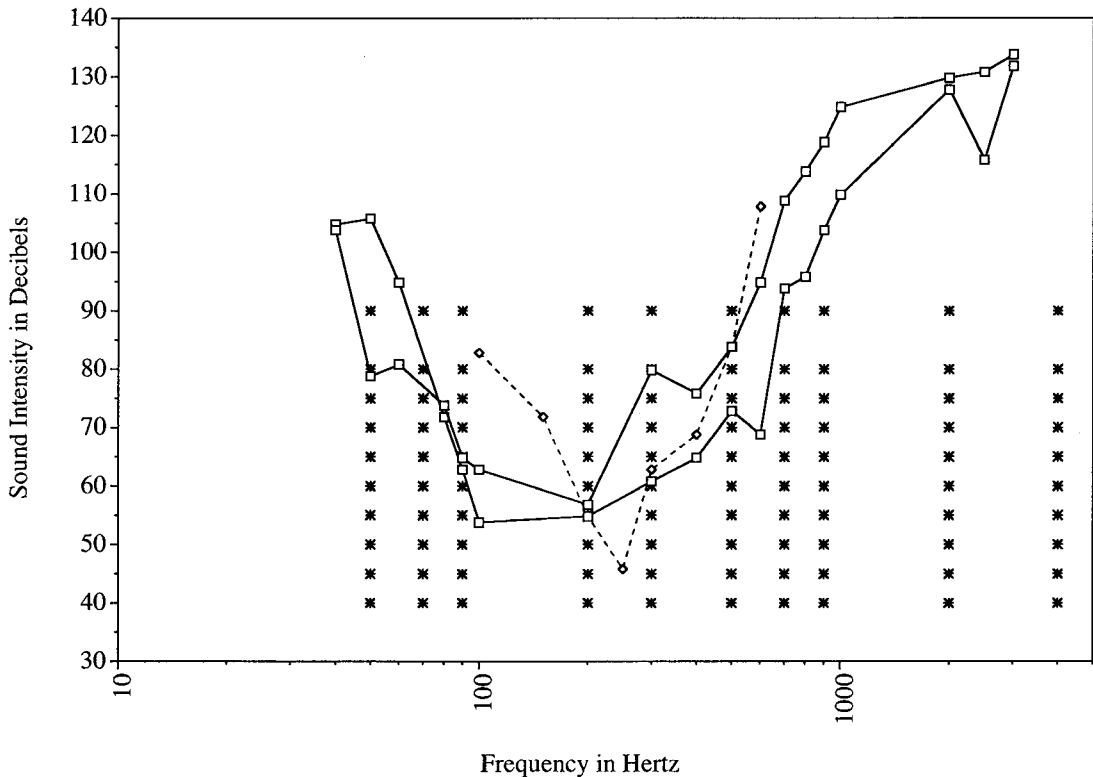


Figure 1. Experimental design of the study. The frequency response curve of *Crotalus viridis* determined by Hartline (1969) is shown with open diamonds and a dashed line; the two curves published by Wever (1978) are shown with open squares and solid lines. The amplitudes of both curves have been converted into standard pressure levels (SPL) with reference to  $20 \mu$  Pascals. The experimental tones synthesized for this study are indicated with asterisks. Only experimental tones within (above) the frequency response curves would be expected to produce positive behavioral responses.

(without the frequency or amplitude modulation used by Young and Aguiar, 2002), and 3) determine the congruence between the range of behavioral responses and the auditory sensitivity curves produced by Wever (1978) and Hartline (1969). The results should provide for a much broader conceptualization of hearing in snakes.

## Materials and Methods

### Live Animals

Ten adult (SVL range 62–73 cm) specimens of the Northern Pacific rattlesnake (*Crotalus viridis oreganus*) were used for this study. All specimens were long-term (> 5 years) captives that had been collected in Whitman County, WA. The rattlesnakes were maintained at 27–31°C, with a 12:12 light cycle, water *ad libitum*, and a diet of pre-killed rodents. Maintenance and use of these animals followed guidelines for reptiles. All experimen-

tal protocols were approved by the Institutional Animal Care and Use Committee of Washington State University.

### Acoustic Stimuli

To explore the behavioral significance of the auditory sensitivity curves produced by Hartline (1969) and Wever (1978), 100 different trial tones, ranging from 50–4,000 Hz, and from 40–90 dB were created (Fig. 1). Trial stimuli were synthesized using SoundEdit16 (Macromedia) running on a G4 Powerbook (Apple); each trial stimulus was a pure tone (having neither temporal patterns, amplitude modulation, nor frequency modulation) with a duration of 20 sec. Once the tone had been synthesized the tone was adjusted to achieve the desired amplitude ( $\pm 0.2$  dB) when measured from the center of the testing arena with a sound level meter (Brüel & Kjaer model 1625). In addition to these 100 trial stimuli, two control stimuli were

synthesized; one was 20 sec of silence, and the second was a pure tone with a frequency of 6,000 Hz and an intensity of 60dB.

### Experimental Design

All trials were conducted within a specially constructed quiet room. A table was placed near the middle of the room and its surface covered with vibration dampening pads (Fisher). A glass-fronted wooden cage (48cm x 57 x 48 tall) rested on top of the vibration dampening pads. Another layer of vibration dampening pads was placed on the floor of the cage, in turn, these pads supported a large steel plate. The mass of the steel plate (16.1 kg), combined with the layers of the vibration dampening pads, prevented groundborne vibrations from contaminating the trials. A Pioneer B20GR30-51F-Q speaker (frequency range 31–7,000 Hz) was suspended from the roof of the cage by a series of loose rubber grommets designed to minimize transmission of vibrations from the speaker to the wooden cage.

The cage was continuously illuminated by a 150-watt light positioned in front of the cage. A digital video camera (DCR-TRV17, Sony) was positioned in the front of the cage at an angle that permitted coverage of the entire steel plate. Behind the video camera a two-layer screen was erected from floor-to-ceiling and wall-to-wall. The researchers remained behind this screen throughout the trials. A video recorder and monitor (coupled to the video camera) used to monitor the rattlesnake and record the data were located behind the screen, as was the computer used to present the stimuli.

Each unique combination of frequency and amplitude was placed on a poker chip. Before each trial nine chips were drawn blindly, as was one of the two chips denoting a control tone. The sequence of these 10 stimuli (nine trials and one control) was then randomized. Each rattlesnake was placed in the trial chamber individually. Trials were only conducted if the rattlesnake was near the center of the steel plate, no data were collected if the specimen was in direct contact with the wooden sides of the trial chamber. The initial handling, and the close proximity of the researchers, provoked rattling in every specimen. Once rattling had commenced the researchers withdrew behind the screen and observed the rattlesnake on the video monitor.

The initial (post-handling) cessation of rattling was used as a behavioral indicator to equilibrate, as much as possible, the behavioral state of each specimen. Trial stimuli were presented 60 sec following the cessation of rattling. The rattlesnake's behavior was recorded for 30 sec immediately prior to the onset of the trial stimulus. In this way both in-series (the 30 seconds prior to each trial tone) and in-parallel (the drawn controls of silence or 6,000 Hz tone) controls were employed. When the trial tone (or in-parallel control) stimulus ceased, the rattlesnake was again induced to rattle by the visual presence of a researcher and the data collection sequence repeated. Each data collection trial lasted approximately 35 minutes. Each specimen was used for only a single data collection series.

### Data Analysis

The data analysis protocols used are similar to those used in earlier experimental studies of snake audition (Young and Aguiar, 2002; Young, 2004). The prevalence of four behavioral markers was quantified from the last 20 seconds of every in-series control period as well as the entire duration of every trial tone (or in-parallel control). The four markers were the number of tongue flicks, the duration of rattling behavior, the incidence of head jerks (the rapid lateral swipes of the head), and the incidence of freezing behavior (which we defined as at least 5 seconds of no motion immediately following sustained locomotor and/or cephalic movements). Rattling, head jerks, and freezing were never observed during the in-series control period, although the rattlesnakes did tongue flick during this time. We scored the rattlesnake as exhibiting a positive response if, during the trial tone, it exhibited either a head jerk, freeze, rattling, or a change of at least two tongue flicks (when compared to the in-series control). Typically, the positive responses were combinations of these four behaviors. The video record of the behavioral trials was scored independently by both authors.

## Results

### General Observations

Overall, the specimens exhibited moderate levels of behavioral activity during the trials. Once placed within the experimental chambers, most of the rattlesnakes maintained a coiled posture, with only

intermittent rattling, throughout the behavioral trials. A few of the specimens initially showed “typical” exploratory behavior before adopting a coiled posture. When positive responses were observed (see below) they were of relatively short duration (e.g., only a few seconds of rattling rather than rattling bouts that were sustained beyond the presentation of the trial tone).

### Results of the Trials

The four behavioral features associated with a positive response — change in tongue flick rate, freezing, head jerks, and rattling — were not observed during presentation of either of the in-parallel controls (silence or 6,000 Hz at 60 dB) both of which were presented during five trials. Twelve of the trial tones fell within the auditory range of *Crotalus viridis* determined by Hartline (1969); a random sample over 90 experimental trials would be expected to produce 10.8 positive results. In contrast, 24 of the trial tones fell within the auditory rang of *C. viridis* determined by Wever

(1978); a random sample over 90 experimental trials would be expected to produce 21.6 positive results. The 90 experimental trials resulted in 30 positive responses, with every specimen exhibiting at least one positive response. The number of positive responses is significantly greater than expected for either auditory sensitivity curve: compared to the predictions from Hartline (1969)  $\chi^2 = 38.78$ ,  $df = 1$ ,  $P < 0.01$ ; compared to the predictions from Wever (1978)  $\chi^2 = 4.30$ ,  $df = 1$ ,  $P < 0.05$ .

### Discussion

The higher than expected number of positive responses (for both sensitivity curves) could be explained in one of two ways; either an unusually high sampling (given the random design) of experimental tones fell within the auditory sensitivity curves, or behavioral responses were found outside of the physiologically-established sensitivity curves (Hartline, 1969; Wever, 1978). The distribution of the positive results (Fig. 2) clearly supports the latter explanation in that most

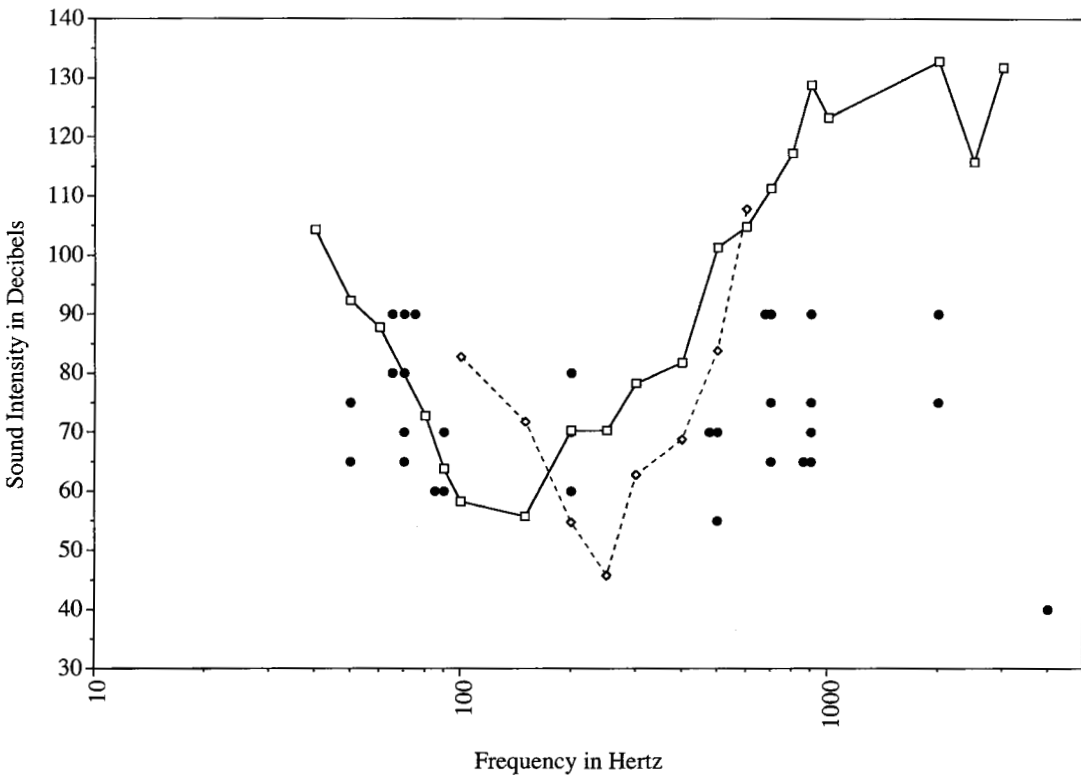


Figure 2. The experimental tones which evoked positive behavioral responses (solid circles) are plotted over the frequency response curves determined by Hartline (1969, open diamond, dashed line) and the mean of the two curves published by Wever (1978, open square, solid line).

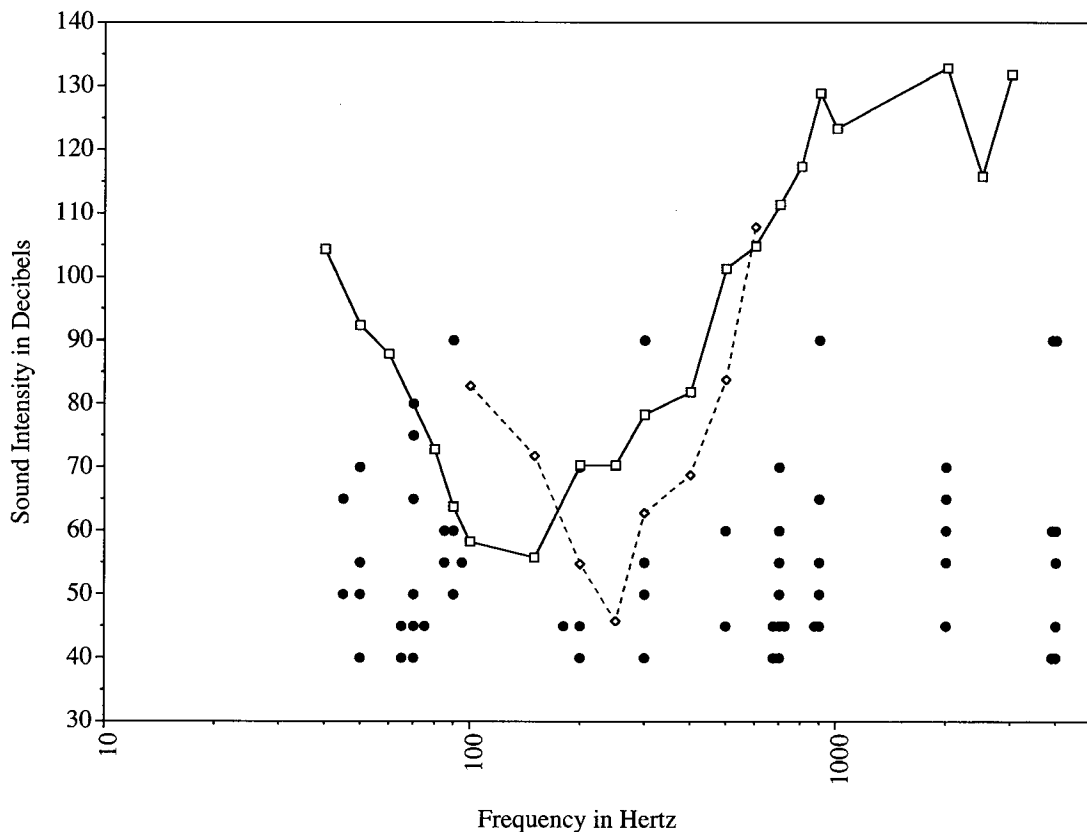


Figure 3. The experimental tones which yielded negative behavioral responses (solid circles) are plotted over the frequency response curves determined by Hartline (1969, open diamond, dashed line) and the mean of the two curves published by Wever (1978, open square, solid line).

of the positive trials fell outside of the sensitivity curves. A number of positive results were obtained from trials at both the low-frequency and high-frequency sides of the curve (Fig. 2). The distribution of these points suggest that the peak sensitivity established by Hartline (1969) and Wever (1978) correspond to the behavioral responses of *Crotalus viridis*; with one exception, no experimental tone presented at less than 55 dB evoked a positive response. The one exception (an experimental tone at 4,000 Hz and 40 dB) represents a distinct outlier both in terms of frequency and amplitude. We suspect that the rattlesnake in this trial was responding to one of the researchers moving at the edge of the screen. Repeated trials at the lower amplitude range (40–55 dB) yielded negative results (Fig. 3).

There is an inherent limitation in studying the sensory system using behavioral evidence. While

the positive behavioral responses provide solid evidence of the underlying sensory basis, negative results must be interpreted with caution. Simply put, the snake may perceive the experimental tone but not exhibit an overt behavioral response. Previous studies (Moore et al., 2000; Secor et al., 2000) have shown that experimental stimuli can evoke significant changes in physiological systems (such as heartbeat) in snakes. A multi-faceted experimental design, that incorporated both behavioral and physiological measurements, could result in stronger agreement in the distributions of negative and positive results (Figs. 2, 3).

Previous studies of audition in rattlesnakes have shown neurophysiological responses to pure tones (Hartline, 1969; Wever, 1978) and behavioral responses to complex tones (Young and Aguiar, 2002; Young, 2004). The current study — which used experimental tones similar to those of Hartline

(1969) and Wever (1978), and reported behavioral responses similar to those of Young and Aguiar (2002) and Young (2004) — suggests that these earlier works can be comfortably integrated. These results further support the idea that crotalids, and presumably other snakes, can respond to a wide range of sounds produced in nature (Young, 2003). The broader frequency response range suggested by the results of this study is restricted to relatively high amplitude (> 60 dB) tones.

Overall, the *Crotalus viridis oreganus* used in this study seemed to have “weaker” behavioral responses than did the *C. atrox* used in the earlier studies (Young and Aguiar, 2002; Young, 2004). In *C. v. oreganus* the most common behavioral response was a change in tongue flick rate. Although this same behavioral response was observed in *C. atrox*, in that species almost all of the positive responses included rattling. Several factors may account for the weaker behavioral response observed in *C. v. oreganus*, when compared to

*C. atrox*: 1) the *C. v. oreganus* were long-term (> five year) captives whereas the *C. atrox* were wild-caught; 2) the *C. atrox* were studied within a chamber designed to eliminate all but controlled auditory cues, the specimens in the present study could still gather visual stimuli through the glass front of the cage; and 3) *C. v. oreganus* is generally described as more “calm” than the excitable and irritable *C. atrox* (e.g., Ernst, 1992; Bartlett and Tennant, 2000). The same behavioral responses were observed in both *C. atrox* and *C. v. oreganus*, despite the fact that these two species are not closely related within the crotalid radiation (e.g., Murphy et al., 2002), suggesting that these behaviors may be a common crotalid attribute.

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