Maturation of the auditory system in clinically normal puppies as reflected by the brain stem auditory-evoked potential wave V latency-intensity curve and rarefaction-condensation differential potentials

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Objective—To evaluate auditory maturation in puppies.

Animals—Ten clinically normal Beagle puppies.

Procedure—Puppies were examined repeatedly from days 11 to 36 after birth (8 measurements). Click-evoked brain stem auditory-evoked potentials (BAEP) were obtained in response to rarefaction and condensation click stimuli from 90 dB normal hearing level to wave V threshold, using steps of 10 dB. Responses were added, providing an equivalent to alternate polarity clicks, and subtracted, providing the rarefaction-condensation differential potential (RCDP). Steps of 5 dB were used to determine thresholds of RCDP and wave V. Slope of the low-intensity segment of the wave V latency-intensity curve was calculated. The intensity range at which RCDP could not be recorded (ie, pre-RCDP range) was calculated by subtracting the threshold of wave V from threshold of RCDP.

Results—Slope of the wave V latency-intensity curve low-intensity segment evolved with age, changing from (mean ± SD) –90.8 ± 41.6 to –27.8 ± 4.1 µs/dB. Similar results were obtained from days 23 through 36. The pre-RCDP range diminished as puppies became older, decreasing from 40.0 ± 7.5 to 20.5 ± 4.1 dB.

Conclusion and Clinical Relevance—Changes in slope of the latency-intensity curve with age suggest enlargement of the audible range of frequencies toward high frequencies up to the third week after birth. Decrease in the pre-RCDP range may indicate an increase of the audible range of frequencies toward low frequencies. Age-related reference values will assist clinicians in detecting hearing loss in puppies. (Am J Vet Res 2000;61:1343–1348)

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Evolution of the audible range of frequencies is a key process in maturation of the auditory system that has not been investigated in dogs. Recording of brain stem auditory-evoked potentials (BAEP) is a simple, noninvasive, and widely available method. It is used in humans to evaluate partial hearing loss, especially for high frequencies. Using responses to intensities that vary from threshold to 90 dB normal hearing level (NHL) makes it possible to calculate the slope of the wave V latency-intensity curve. Moreover, the difference between waves evoked by rarefaction and condensation click stimuli (ie, rarefaction-condensation differential potential [RCDP]) also can be used. Slope of the wave V latency-intensity curve and the range of stimulus intensity at which RCDP cannot be recorded are used to evaluate high-frequency hearing loss in humans and, thus, to indirectly evaluate the audible range of frequencies.

Maturation of BAEP in dogs has been investigated, but that latency study was restricted to high-stimulus intensities (75 to 105 dB NHL). To our knowledge, RCDP has not been evaluated in dogs.

In the study reported here, our objective was to use the wave V latency-intensity curve and RCDP to provide an initial estimate of the evolution of the audible range of frequencies in growing puppies. Furthermore, we intended to determine age-related reference values for these 2 variables.

Materials and Methods

Animals—Ten Beagles (5 females and 5 males) were randomly selected from 3 litters. Measurements were repeatedly obtained at 3-day intervals from day 11 to day 29 after birth; a final measurement was obtained on day 36. Puppies were not sedated or anesthetized for recording sessions. Recordings were collected during periods of natural sleep. The experimental protocol was approved by the local ethics committee. Puppies were cared for in accordance with principles established in the NIH Guide for Care and Use of Laboratory Animals.

Recordings—An electrodiagnostic system was used to obtain recordings. Differential recordings were obtained, using stainless-steel needles inserted subcutaneously in the vertex of the skull and the area of the mastoid, ipsilateral to the stimulated ear. The vertex electrode was connected to the noninverting input of the preamplifier, and a positive value recorded by this electrode was displayed as an upward deflection. An additional needle was inserted at the base of the neck and served as a ground electrode. Impedance of the
Click repetition rate was 21.7 Hz. A contralateral band limiting masking noise (20 Hz to 20 kHz) was delivered at 40 dB less than the value for the click stimulus. Intensity of contralateral masking noise was delivered at 40 dB less than the value for the click stimulus. Intensity of contralateral masking noise was delivered at 40 dB less than the value for the click stimulus. Intensity of contralateral noise (20 Hz to 20 kHz) was delivered at 40 dB less than the value for the click stimulus. Intensity of contralateral noise (20 Hz to 20 kHz) was delivered at 40 dB less than the value for the click stimulus. Intensity of contralateral noise (20 Hz to 20 kHz) was delivered at 40 dB less than the value for the click stimulus.

A foam eartip of the proper diameter was fitted in the opening of each ear canal. From days 11 to 17 after birth, it was impossible to introduce the eartip into either auditory canal. The extremity of the silicon tubing was glued to the opening of the ear canal, using silicone paste, and the tubing was secured to the pinna, using adhesive tape.

Figure 1—Representative recordings of brain stem auditory-evoked potentials (BAEP) in a young puppy. For each stimulus intensity (90 dB normal hearing level [NHL] in this figure), responses to rarefaction (Rar) and condensation (Cond) click stimuli were added (R+C) or subtracted (R–C, top tracings). A reference tracing (R, 90 dB NHL) was made before and after each measurement session (bottom). Scale in microvolts (µV) and milliseconds (ms).

Figure 2—Representative BAEP recordings for R+C (left) and R–C (right) in a 29-day-old puppy. Stimulus intensity is indicated on the left. The threshold intensity for R+C and R–C is indicated (arrow). Recordings for –6, 0, and 20 to 35 dB were repeated. 1 = Wave I, V = Wave V, M = Microphonic cochlear potential. Time scale: 3 milliseconds (ms); amplitude scale in microvolts (µV) on the right.
and the intensity for which a signal was not detected (Fig 2). However, for 22 recordings, the threshold of wave V was less than the lowest stimulus used (–6 dB NHL). In these puppies, the threshold of wave V was arbitrarily assigned a value of –7.5 dB NHL.

Mean absolute difference of mean latency-intensity curve was 3.4 μs/dB (mean value of –28 μs/dB, a mean relative error of 12%). The threshold of wave V varied from 0 to 5 dB, whereas the threshold of RCDP varied from 0 to 15 dB, and the pre-RCDP range varied from 5 to 10 dB. For the data obtained during the 76 sessions, the regression line fitted to the low-intensity segment of the wave V latency-intensity curve was calculated from 0.7 ± 1.3 points, and the adjusted correlation coefficient ($r^2$) was 0.92 ± 0.07.

Statistical analysis—Variables considered were slope of the low-intensity segment of the wave V latency-intensity curve, threshold of wave V, threshold of RCDP, and pre-RCDP range. For each variable, homogeneity of variance among age classes was tested, using a Bartlett test. Because the variances were not homogenous, values for each age class were compared with values for day 36, using nonparametric tests for paired data (Wilcoxon test). Latencies for waves I and wave V at a stimulus intensity of 90 dB, and the difference in latency between wave V and wave I also were compared in the same manner. Significance was set at a value of $P < 0.05$.

Missing data (n = 4 out of 80 measurements) were replaced by the arithmetic means of values for adjacent ages. Threshold of RCDP and pre-RCDP range for day 11 were excluded from comparisons, because threshold of RCDP was observed in only 3/10 puppies. The RCDP threshold was not observed in 1/10 puppies on day 14; therefore, the lowest plausible value (97.3 dB) was used for comparisons.

Within each age class and for each variable, the effect of sex was investigated by use of Student t-tests. Similarly, effect of litter for each age class and variable was examined by use of an ANOVA.

Results

Results were reported as mean ± SD. Sex or litter of the puppies did not affect the 4 variables. Tracings for each puppy were evaluated, and threshold values were determined (Fig 2).

Slope of the low-intensity segment of the wave V latency-intensity curve became shallower as puppies became older, evolving from –90.8 ± 41.6 μs/dB on day 11 to –30.2 ± 2.3 μs/dB on day 23 (Fig 3); values then remained constant until day 36 (Fig 4). In a similar manner, the threshold of wave V decreased from 62.5 ± 24.6 dB NHL on day 11 to –4.5 ± 2.6 dB NHL on day 20; values then remained constant until day 36.

Threshold of RCDP diminished from 72.8 ± 19.9 dB NHL on day 14 to 23.5 ± 6.1 dB NHL on day 29, and it then remained constant until day 36. In all pup-
pies on day 11 and 1 puppy on day 14, a threshold for RCDP could not be detected (ie, we did not detect a difference between responses to rarefaction and condensation stimuli in the intensity range used [threshold to 90 or 95 dB]; Fig 4).

The pre-RCDP range diminished throughout the study as puppies became older. The range decreased from 40.0 ± 7.5 dB on day 14 to 20.5 ± 6.4 dB on day 36 (Fig 4).

At 90 dB NHL, latency of wave V decreased significantly (P = 0.01) from 7.84 ± 1.14 to 4.32 ± 0.11 milliseconds. Similarly, latency of wave I decreased significantly (P = 0.01) from 2.34 ± 0.11 to 1.80 ± 0.06 milliseconds, and the difference in latency of wave V and latency of wave I decreased significantly (P = 0.01) from 4.16 ± 0.24 to 2.52 ± 0.09 milliseconds.

**Discussion**

Slope of the latency-intensity curve is steeper in humans with high-frequency hearing loss, compared with clinically normal humans. The pre-RCDP range also is reduced in those people. In humans with conduction hearing impairment, an increase in the threshold of wave V and an unchanged slope in the latency-intensity curve is observed.

A steeper slope of the wave V latency-intensity curve in high-frequency hearing loss is explained by a change of the frequency-tuning curve of affected auditory units. The frequency-tuning curve of an auditory unit represents the threshold intensity as a function of the frequency. This curve is highly asymmetric for high-characteristic frequency units. There is a sharp tip (ie, a low threshold) in the curve at the characteristic frequency and a broad tail with a higher threshold extending toward lower frequencies. In hearing loss, the tip-to-tail ratio is diminished or abolished.

In this case, the stimuli reach the thresholds of high-frequency units only at high-level stimuli, allowing similar latencies to be recorded. At low-level stimuli, the stimulus is less than the thresholds of high-frequency fibers, and only the lower-frequency region contributes to the recorded responses. The lower the characteristic frequency, the longer the latency, because the low-frequency-sensitive area of the cochlea is more apical and is reached later by mechanical events that travel along the scala vestibuli. The steeper slope of the wave V latency-intensity curve reflects these latency changes.

In kittens, the threshold of the frequency-tuning curve matures progressively during the first to third week after birth. However, the progress is much larger at the tip than at the tail, resulting in an increase in the tip-to-tail ratio. Flattening of the slope of the wave V latency-intensity curve between days 11 and 20 in the study reported here may originate from a similar process, with near-threshold stimuli recruiting progressively more basal units as the tip matures.

The low-threshold sharply tuned tip observed in the tuning curves of the high-characteristic frequency units is believed to result from collaboration between outer and inner hair cells. At low-stimulus intensities, which need this collaboration to build up responses in the inner hair cells, differences between the responses to rarefaction and condensation stimuli have not been observed. The range of intensities for which RCDP cannot be observed decreases or is abolished in high-frequency hearing loss. The earliest event in high-frequency hearing loss is an increase of the threshold and a diminution of the tip-to-tail ratio of the involved
auditory units. This diminution in the tip-to-tail ratio supposedly causes a reduction in the pre-RCDP range.

The pre-RCDP range decreased with increasing age of the puppies reported here. This seems to be in contradiction with the argument for the slope of the wave V latency-intensity curve pointing toward a maturation of the tip-to-tail ratio of the high-frequency tuning curves. Technical limitations of the measurements precluded an accurate measurement of the threshold of wave V (arbitrarily set at −7.5 dB NH) in some dogs after day 20. This may have artificially diminished the pre-RCDP range in the oldest puppies. Possible filtering properties of an immature ear canal also should be considered. Small volume and high compliance of the ear canal may favor the high-frequency content of a stimulus. However, maturation of the threshold of low-characteristic frequency auditory units may contribute. Low-characteristic frequency units do not have the asymmetric aspect indicative of high-characteristic frequency units. Such units are not believed to provide identical responses to rarefaction and condensation stimuli even at near-threshold intensities. Consequently, maturation of the threshold of low-characteristic frequency units may contribute to a decrease of the RCDP range with increasing age. The audible range of frequencies in kittens enlarges, mostly toward high frequencies but also toward low frequencies, until the third week after birth.

In the study reported here, an intensity range without a RCDP existed after day 11 in all puppies. This is in agreement with studies in which it was reported that tuning curve tips exist in cats as early as the first week after birth, and that these tips may be as sharp as those in adult cats.

In high-frequency hearing loss, an increase in threshold of wave V is observed in combination with a steeper slope of the wave V latency-intensity curve. The increase in threshold of wave V before day 14 could be caused, in part, by immaturity of the external and middle ear, which can attenuate stimuli. However, immaturity of the cochlea and retrocochlear structures may account for the increased threshold of wave V in puppies that are 14 to 20 days old. Puppies that are 11 days old will have a sealed ear canal, attenuating the stimulus intensity that reaches the inner ear and shifting the wave V latency-intensity curve to the right but leaving its slope unchanged. In the same conditions, the threshold of wave V and threshold of RCDP are increased, but their difference (ie, pre-RCDP range) remains unchanged.

Variables examined in the study reported here had a larger variance in the youngest puppies. Possible effects of sex or litter were not detected. Such possible effects may have been canceled by measurement error in the lower-intensity range.

The threshold value of wave V reached by day 20 in this study is similar to values reported in adult dogs. However, values reported for adult dogs in other studies are slightly or extremely higher.

The age-related reference values provided in this study for threshold of wave V, low-intensity segment of the wave V latency-intensity curve, and pre-RCDP range may assist clinicians when making a diagnosis of deafness in young puppies and when studying the progress of hereditary cochleosaccular deafness that may take place in puppies of the age range included in our study.

Decrease of the latency of wave V and decrease in the difference between latency of wave V and latency of wave I (measured with a stimulus of 90 dB) reflects maturation of the neural auditory pathways with improvement of synaptic efficiency and conduction velocity. This evolution proceeds beyond the age of dogs included in our study.

References


