MRI measurement of the canine auditory pathways and relationship with brainstem auditory evoked responses

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Summary

The objective of this study was to determine direct measurements of auditory pathways by magnetic resonance imaging (MRI) during the growth period of healthy Beagles, and to discover how canine brainstem auditory evoked response (BAER) latencies vary in relation to these MRI measurements. Eighty healthy Beagles were tested at eight, 16 and 52 weeks of age (stages 1, 2, 3, respectively) with BAER and brain MRI. The BAER interpeak latency (IPL) II-V and brain MRI neural generators of BAER waves II and V were identified. A linear distance was calculated in millimeters in order to determine the approximate length of auditory pathways. Sensory nerve conduction velocity (SNCV) of the auditory pathway between peak II and peak V was calculated for each group. A significant difference was observed between brain MRI distances among the three stages. Mean BAER IPL II-V were not significantly different between the three stages. The progressive growth of the skull and brain witnessed by the progressive increased distance of the MRI auditory pathways between peak II and peak V was not associated with a progressive maturation of the BAER IPL II-V. The SNCV of the auditory pathway between peak II and peak V was 6.14 m/sec for group 1; 6.76 m/sec for group 2; and 7.32 m/sec for group 3.

Introduction

Brainstem auditory evoked response (BAER) is an averaged, time-locked recording of the electrical activity that travels along the auditory pathways of the brain in response to an externally applied acoustic stimulus (1). Electrical stimuli are recorded by subdermal electrodes that are positioned in specific locations along the skull, and responses are summated by a computer and averaged in order to extract a signal from extraneous background electrical noise (2).

Animal studies have attempted to identify the neural generators for each peak of the BAER waves. Based on pathological and clinical data, lesion studies, and a comparison of surface and depth recordings, there is general concensus that wave I originates at the ipsilateral cochlear nerve, wave II corresponds to the ipsilateral cochlear nucleus and unmyelinated central terminals of the cochlear nerve, wave III originates from the dorsal nucleus of the trapezoid body (ipsilateral and/or contralateral), and wave V is generated in the ipsilateral and/or contralateral caudal colliculus (1, 3–10). The neural generators for waves IV, VI and VII remain undefined in the dog (1, 2, 7, 10, 11).

In humans, significant positive correlations have been found between the external measurement of the head and BAER latencies, which suggests that the length of the neural pathway can be correlated to the size of the head and cranial vault (12–14).

In veterinary medicine, a positive correlation was also found between external measurements of head and BAER latencies in healthy control dogs (15, 16). Another study investigating Dalmatians and Jack Russell Terriers of the same age found a significant difference in BAER latencies between the two breeds although an intra-individual variation within each breed was not found over time despite a progressive increase in skull size noted within each breed. These results indicated that the breed of the dog might play a more significant role in the obtained BAER measurements than the actual head size (17).

A limitation of these human and veterinary studies is the indirect method used to calculate the length of the neuronal auditory pathways by manual measurement of the external head size (head circumference). To date, MRI measurements of the brain stem have only been used in one human study in order to assess the variability in BAER. It was found that significant positive correlations existed between the actual length of the brain stem and the I-V and III-V interpeak latencies (18). This study also revealed that this correlation between the length of the brainstem and BAER interpeak latencies was most significant during the period of growth of the skull (18). To the best of the authors' knowledge such information is not available in the veterinary literature. Therefore, the purpose of the present study was to determine direct measurements of MRI auditory pathways during the growth period of healthy Beagles from eight to 52 weeks of

age and to investigate how canine BAER latencies vary in relation to measurements of MRI auditory pathways.

Materials and methods

Study population

Eighty healthy Beagles were selected for the study. The study was conducted at the Ontario Veterinary College, University of Guelph, Canada, and was performed in accordance with the guidelines and upon approval of the Animal Care Committee of the University of Guelph. Each dog was enrolled at the age of eight weeks and was followed throughout the maturation period until 52 weeks of age. Each dog was tested over time at three different maturation stages: eight, 16 and 52 weeks. Three groups were created based on the different maturation stage: group 1 = 8 weeks of age; group 2 = 16 weeks of age, and group 3 = 52 weeks of age. Each dog received the same testing protocol during the three stages of maturation which consisted of physical and neurological examinations, BAER test and brain MRI. Therefore, each dog had three physical and neurological examinations, three BAER, and three brain MRI over time. The data for each dog were analyzed if the following inclusion criteria were fulfilled: 1) Acceptable BAER recordings for each dog and readable traces bilaterally during the three stages of maturation; 2) Acceptable brain MRI images for each dog during the three stages of maturation. If bilateral or unilateral deafness was observed, or if BAER recordings and MRI measurements were not consistently reproducible, the data were not analyzed. This study was part of a larger investigation sponsored by the CanCog Technologies Inc., Toronto, ON, Canada).

BAER recording

Each dog was sedated with a standard protocol (acepromazine[a] 0.3 mg/kg and hydro-

morphone[b] 0.1 mg/kg IV) in order to ensure acceptable recordings by minimizing artifacts generated by muscle movements (2). The extent of sedation was assessed based on the degree of muscle relaxation, prolapse of the nictitating membrane and observed mental tranquillity of the animal. Adjustments to the sedation protocol were made as necessary in order to ensure effective recording with minimal interference from the animal. The recordings were obtained by using an electrodiagnostic system (Cadwell model Excel, serial No. 081290175, Cadwell Laboratories Inc, Kennewick, WA, USA). Each dog was positioned in sternal recumbence and three needle electrodes were placed subdermally for the BAER recording. The recording electrode was placed at the vertex of the skull, the reference electrode was placed just rostral to the tragus of the tested ear and the ground was positioned just rostral to the tragus of the non-tested ear. The ear canals were examined to check that they were free of debris and otherwise unobstructed in order to ensure that the stimulus was delivered correctly. Internal foam ear plugs were placed in each ear canal and 'clicks' were chosen as the preferred stimulus in order to provide the largest response amplitude for each recorded waveform. Repetitive alternating stimuli were delivered to the tested ear through the internal ear plug at a rate of 11 clicks per second at sound intensities that progressed from 60 to 90 dB hearing level. An average recording of 1000 sweeps for each ear of each dog was recorded and was stored for later measurement and analysis. Each animal was closely monitored by a reg-

istered veterinary technician throughout the entire duration of the procedure.

BAER measurements

BAER latencies were calculated by use of manually directed cursors on the oscilloscope (Fig. 1). Latencies were measured in milliseconds (msec) and were calculated as the interval from the onset of the stimulus to the positive peak of the waveform. BAER interpeak latencies (IPL) II-V were calculated. The IPL II-V, also known as the central conduction time, corresponds to the approximate time required for the electrical activity generated in the cochlear nucleus to reach the mesencephalon. Mean BAER IPL II-V were calculated on each side (left and right) for all three groups.

Brain MRI recording

Following BAER recording, each dog was anaesthetized in order to have a brain MRI. A standard anaesthetic protocol was used which consisted of induction with propofol[c] (0.06 mg/kg IV) and maintenance with isoflurane[d]. Each dog was then placed in a 1.5 Tesla whole-body MRI system (GE Healthcare, Waueska, WI, USA)where transverse, sagittal and dorsal T1-weighted images of the brain were acquired with a 3-D-FSPGR (fast spoiled gradient recalled echo) pulse sequence with an inversion preparatory pulse (TR:10.6 msec; TE:4.7 msec; TE:175
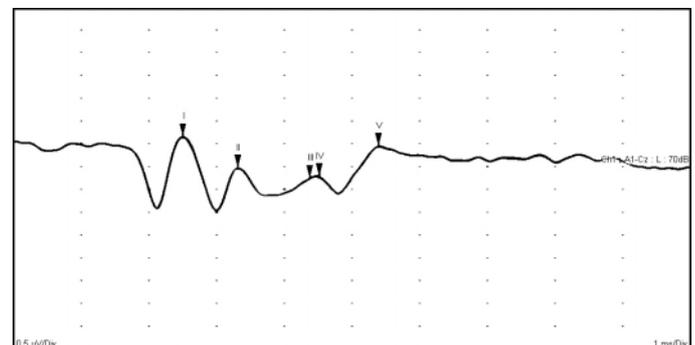
Fig. 1
Brain auditory evoked response demonstrating the location of peaks II and V.

msec). The image resolution was 0.625 mm in plane with a slice thickness of 1 mm, with no interslice interval.

Brain MRI measurements

Neural generators of wave II (cochlear nucleus) and wave V (caudal colliculus) of the BAER waveforms were identified on transverse T1-weighted images ipsilaterally along the auditory pathway. A linear distance expressed in millimeters (mm) was calculated between the cochlear nucleus and the caudal colliculus by using a software program for tridimensional visualization of the brain developed by the Massachusetts Institute of Technology (3D-Slicer version 2.4© 2004 MIT, Massachusetts Institute of Technology, www.slicer.org). The other neural generators were not measured due to the lack of consistency in reproducing the neuroanatomical structures on MRI evaluations. An oblique projection of the brain was created with an AW software program (Advantage Workstation version 4.2. GE Healthcare, Waueska, WI, USA) in order to display the distance between the cochlear nucleus and the caudal colliculus in the same plane (Fig. 2).

Sensory nerve conduction velocity of auditory pathway II-V

Sensory nerve conduction velocity of the auditory pathway between the cochlear nucleus (peak II) and the caudal colliculus (peak V) was calculated for each group by dividing the mean values of the linear distances between peak II and peak V by the mean value of BAER IPL II-V.

Statistical analysis

Statistical analyses were performed using commercially available software (SAS Institute, SAS Online Doc®, version 8, Cary, NC, SAS Institute Inc., 1999). All of the data were reported as mean ± standard error (SE). A Shapiro-Wilk test was performed to check for normality of the data. When required, the data were log transformed in order to improve the distribution. In order to compare the physiologic variables among treatment groups over time, ANOVA for repeated measures with an appropriate covariance structure was performed using time, side, sex, as well as their interaction as factors. If the overall f test was significant for

the main effects, pair wise comparisons were based on a Tukey's adjustment. Pearson correlation analysis was used to determine whether there was an overall association between BAER and MRI, and a MANOVA was performed in order to measure the association between BAER and MRI within the maturation stage (group).

Results

Study population

Twenty-three (nine males and 14 females) out of 80 healthy Beagles met the inclusion criteria and the results were compared longitudinally among the three groups. The remaining dogs (n=57) were excluded from the study based on the following criteria: the inability to record proper BAER testing in one or both of the ears; difficulty in interpreting the exact location of neural generators in BAER recordings; difficulty in identifying and consistently reproducing neural generators in brain MRI images; a lack of consistent BAER and MRI measures intra and inter-individuals over the course of the three maturation stages; and the presence of unilateral or bilateral deafness.

BAER recordings

Mean BAER IPL II-V were 1.87 msec for group 1; 1.91 msec for group 2; and 1.82 msec for group 3. The differences between groups were not significant (Table 1).

Brain MRI analyses

Neural generators of BAER recording identified consistently along the MRI auditory pathway included the cochlear nucleus for peak II and the caudal colliculus for peak V bilaterally. Linear distances expressed in millimeters (mm) calculated ipsilaterally between these structures were determined for each dog at each maturation stage (eight, 16, and 52 weeks). The means values of the linear distances between peak II and peak V for each group were: group 1=11.49 mm; group

2=12.92 mm; and group 3=13.34 mm. A statistical significant difference was observed between the three groups (P ≤ 0.05) which indicates that the progressive increase in distance between peak II and peak V is associated with progressive growth of the brain and head observed over time (Table 1).

Table 1
Statistical significant difference of the mean MRI distances between neural generators of peak II and V among the three groups.

Group	Mean BAER IPL (ms)	Mean MRI distances (mm) between neural generators of peak II and V
Group 1	1.87[a]	11.49[b]
Group 2	1.91[a]	12.92[c]
Group 3	1.82[a]	13.34[d]

[a-d] Within each column, values with different superscript are significantly (*P* ≤ 0.05) different. IPL: Interpeak latency.

Sensory nerve conduction velocities of auditory pathway II-V

Sensory conduction velocity (SNCV) of the auditory pathway between the cochlear nucleus (peak II) and the caudal colliculus (peak V) was calculated for each group and was expressed in metres per seconds (m/sec). The SNCV was 6.14 m/sec for group 1; 6.76 m/sec for group 2; and 7.32 m/sec for group 3, and differences between groups were not significant (Table 1).

Relationship between BAER and MRI recordings

Overall, a linear relationship was not observed over time between mean BAER IPL II-V and mean brain MRI distances of the auditory pathways (*P* = 0.178). These findings suggested that the progressive growth of the skull and brain deduced by the progressive increased distance of the MRI auditory pathways between peak II and peak V was not associated with being a progressive maturation of the BAER IPL II-V. Gender was not found to be a significant factor.

Discussion and conclusions

In this study, the significant difference that was observed in the MRI measurements of the auditory pathway between neural generators of peak II and peak V during the three stages of maturation indicates a progressive increase in skull and brain sizes over time. Although external measurements of the head and brain sizes were not performed in this study, there was strong evidence that the observed MRI measurements of the auditory pathway were directly related to the growth of skull and brain as would be ex-

pected to happen during the maturation period. These results are in agreement with the human study conducted by Antonelli et al. in which direct MRI measurements of the brain stem revealed a progressive increase of the distances over time between the neuroanatomical structures related to peak and interpeak latencies (18). In our study, the minor significant differences that were observed between the distances calculated by MRI measurement during the three stages of maturation were mainly due to the low inter-individual variability of the selected patients since all of the dogs that were enrolled in the study belonged to the same breed (Beagles). Serial calculations of MRI distances in different breeds, including large and small breeds, during the maturation period would have been useful in order to understand the variability of the brain stem growth over time in dogs with different head sizes. Unfortunately, the selected population of our study was controlled by a larger research project performed at the same time and dogs of other breeds were not enrolled.

The calculation of the sensory nerve conduction velocity between peak II and peak V represents a non-invasive and innovative method for the evaluation of the integrity and functionality of the sensory auditory pathways. Normal reference parameters have been generated in the present study. Further studies are warranted in a clinical setting where the sensory nerve conduction velocity may represent a valid method to assess the integrity of the auditory pathway in pathological processes of the central nervous system, such as demyelinating disorders, encephalitis or other parenchymal lesions that predominantly affect the brain stem region.

BAER IPL II-V did not change over time during the three stages of maturation which

concurs with previous results published by Strain et al., indicating that BAER latencies reach maturity by day 40 and that further changes in BAER latencies are not expected from that time onwards (19). The lack of relationship observed over time between mean BAER IPL II-V and mean brain MRI distances of the auditory pathways suggest that the extrapolated progressive growth of the skull and brain is not associated with a progressive maturation of the BAER IPL II-V. Possible mechanisms that explain why BAER IPL remain relatively constant over time while the brain continues to grow in size include the caudal to rostral pattern of maturation seen within the central nervous system. (20) The development of the brain proceeds from brain stem to cortex. As a result, the early BAER peaks, which are generated in the lower brain stem, mature before late peaks which are generated at more rostral levels. Other potential mechanisms are related to the maturation stage, myelination and functional integrity of the auditory pathway assessed by BAER (19). The normal process of maturation of the auditory pathway assessed by BAER recordings is characterized by a progressive appearance over time of different neural generators along the BAER trace. Based on previous studies conducted in healthy Beagles, it was hypothesized that as the ear canal opens at 12–13 days of age, the neural generators of peaks I, II, III, and V became progressively evident on BAER traces (19) Specifically, it was concluded that peak I was the first peak to appear and it was visualized on BAER traces by day 20 followed by progressive visualization of other peaks (II, III, V). Calculated BAER latencies between the onset of the stimulus and different peaks, as well as interpeak latencies, reached adult values by day 40 (19). In our current study, the Beagles that were enrolled in group 1 were

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eight weeks old when assessed for the first time with BAER testing. Since maturation of the auditory pathways is usually complete by this time, the lack of BAER IPL II-V changes that were observed among the three groups is likely to be related to the adult age of our subjects at the time of the BAER testing.

A major limitation of this research project was the study population with regard to breed and age at the time of enrollment. Further considerations and conclusions regarding a possible correlation between BAER latencies and MRI measurements of the auditory pathways during the maturation period could have been improved by selecting younger dogs (below the age of 40 days) of different breeds and with different head sizes.

In conclusion, the direct MRI measurement of the auditory pathways along the brain stem and the calculation of sensory nerve conduction velocity between peak II and V represent innovative and feasible methodologies which add more information and precision on the evaluation of the functional integrity of the auditory pathways. Further clinical studies are warranted in order to investigate the use of these measurements in clinical patients that are affected by intracranial pathologies.

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