

## **Multimodal approaches to hearing testing in veterinary medicine**

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### **SENSE OF HEARING AND ITS ASSESSMENT**

Hearing can be assessed based on information from history, questionnaires, or behavioral tests.<sup>1</sup> Objective hearing testing is well established in veterinary practice as a routine diagnostic test for the diagnosis of unilateral and bilateral genetic deafness. The gold standard for hearing screening for congenital deafness is the recording of brainstem auditory evoked responses (BAER) with click stimuli.<sup>2-6</sup> Impaired hearing can result from genetic deafness, or acquired causes: structural disease of the middle ear, inner ear, auditory nerve, developmental defects of the inner ear and hair cells, and degeneration of the hair cell degeneration due to ototoxicity, acoustic trauma, noise-induced hearing loss or age-related hearing loss (presbycusis) affecting hair cells or central processing of auditory stimuli. Furthermore, alterations in the composition and circulation of the potassium-rich endolymphatic fluid could also influence hearing. Classification of hearing deficits distinguishes between impaired sound conduction from disease of the external ear, tympanic membrane, or middle ear ossicles, and sensorineural hearing deficits, which frequently arise from cochlear disease and less commonly retrocochlear lesions in the auditory nerve. The stapes is closely attached to the oval (vestibular) window and transmits its vibrations to the perilymph, endolymph, and basilar membrane of the organ of Corti. The decreasing stiffness of the basilar membrane from base to apex and the amplifier function of the outer hair cells determine the high frequency resolution and tonotopic organization of the cochlea: High frequencies are perceived close to the base, and low frequencies close to the apex of the cochlea. Loudness and hearing ability are measured in decibels, which is a logarithmic scale. A difference of 20 dB equals a 10-fold increase. Be aware that two different decibel scales are in use. Soundmeters use decibel sound pressure level (dB SPL), and 0 dB SPL equals 20 micropascals. Audiometers use decibel normal hearing level (dB nHL). Zero dB nHL is normalized to the normal hearing threshold of humans for a particular frequency or stimulus. Correction factors are lowest for 4 kHz and higher for lower and higher frequencies, and 25 dB for clicks. Companies may apply additional correction factors to estimate behavioral thresholds (dB eHL).<sup>7,8</sup>

### **CURRENT APPROACHES IN VETERINARY MEDICINE**

Routine hearing screening of puppies with BAER only aims to identify deaf ears with hearing thresholds above 80 dB nHL. Thus, click stimuli are presented at supramaximal stimulation intensities to each ear. Conventional BAER testing for congenital deafness uses 80 dB nHL stimulation intensities, far exceeding threshold levels. Clicks are presented via insert earphones to one ear and a contralateral masking noise is recommended. Shortly, the BAER hearing screening test: Time-linked neural activity is recorded from the skull surface with a simple bipolar montage with subcutaneous needle electrodes. The BAER presents as a series of vertex-positive peaks labelled by convention I, II, III and V (IV is usually missing in dogs). The BAER is a far-field neural response from the auditory nerve and afferent conduction in the brainstem auditory pathway. Problems related to noise from movements of the dog and low amplitude potentials in the range of nV are overcome by an averaging process sampling at least 500 responses or more in the time domain, or until the potential is stable and peaks are consistently and reliably identified. Sedation may be necessary in incooperative dogs and can speed up the sampling process and the quality of the recordings.<sup>9</sup>

The BAER method also offers options for a more detailed audiometric assessment: measurements of hearing thresholds, comparing hearing thresholds between air- and bone-conducted stimuli to assess conduction deafness from middle ear effusions or otitis externa, or to assess hearing thresholds and outer hair cell function in detail over a range of frequencies using tone-burst evoked BAER or otoacoustic emissions for further investigation of age-related hearing loss, noise-induced hearing loss or ototoxicity. Wave V of the BAER indicates auditory sensitivity and hearing threshold, because it can be tracked down to the hearing threshold. However, long examination times, lack of standardization, together with the need for prolonged sedation or anesthesia to reduce movement artefacts limited expanded hearing testing in geriatric and diseased dogs. Next-generation hearing screening methods and portable audiometers from newborn hearing screening could offer solutions to overcome this gap

### **ADVANCEMENTS IN HEARING SCREENING DERIVED FROM NEWBORN HEARING SCREENING**

Newborn hearing screening is routinely applied in babies worldwide, with the aim of identifying and treating hearing impairment as early as possible in a child's life, considering the fatal consequences of impaired hearing for speech development. Newborn hearing screening faces the same challenges as hearing testing in veterinary medicine: to perform hearing tests in a short time span while maintaining quality. In newborn hearing screening, distortion product otoacoustic emissions (DPOAE) serve as initial rapid screening tests. Otoacoustic emissions result from active biomechanical processes in the cochlea. Outer hair cells contract rhythmically and act as cochlear amplifiers, enhancing sound signals and creating an "echo" that travels back into the ear canal. The otoacoustic emission is generated directly by the outer hair cells and is independent of the auditory nerve. The emissions typically have very low amplitude (between -10 and +20 dB SPL). For clinical purposes, evoked OAEs are most relevant. DPOAEs are generated and recorded by a probe in the ear canal, emitting two different frequencies simultaneously. The outer hair cells' nonlinear responses produce distortion products (e.g.,  $2f_1-f_2$ ), measurable as acoustic emissions (tones). DPOAE allows rapid frequency-specific assessment over a range of frequencies relevant for human speech, usually from 1 to 8 kHz. In veterinary medicine, DPOAE has been used for initial hearing screening in puppies and for assessment of outer hair cell function in noise-induced hearing loss and geriatric dogs.<sup>10-12</sup> The examination requires only a specialized probe that includes a microphone and loudspeaker and that is placed into the ear canal. Specific needs for a successful examination are a probe that fits into the ear canal and occludes it tightly, an ear canal without any cerumen or exudate, correct placement of the probe directed towards the tympanic membrane, and a quiet and sound-protected environment. OAEs do not measure hearing thresholds; rather they provide a fail or pass for each tested frequency based on the recorded signal-noise difference.  $F_2/f_1$  levels of 65/55 dB are advantageous in veterinary patients, and thorough ear cleaning and a sound-controlled environment are critical for successful recordings. Any cerumen in the ear canal, or within the probe, and even noise from heavy breathing or a respirator machine, could prevent the recording, but if the placement is successful and environmental conditions are controlled, DPOAE can be obtained within less than a minute.

Auditory brainstem responses (BAER) or auditory steady state responses (ASSR) are requested if the DPOAE recording fails. Both methods present robust confirmatory tests with high specificity and record the neural responses after auditory stimulation either in the time domain (BAER) or the spectral domain (ASSR). Both use next-generation technology (binaural stimulation modes, automated peak V recognition, and defined stop criteria), speeding up the sampling process and saving examination time.

The examination follows a stepwise approach. It is recommended that an initial screening test should identify the hearing threshold for each ear with a broadband stimulus, either a click or a chirp. Chirp stimuli are clicks that are adjusted to the length of the human cochlea with the aim of enhancing peak V of the BAER. A human chirp stimulus was recently applied for rapid hearing screening in dogs with a portable audiometer from human newborn hearing screening using binaural test mode and next next-generation technology with automated peak recognition. Furthermore, clicks were presented with rapid 90 Hz stimulation rates. Hearing thresholds could be measured for both ears within a testing time of less than 2 minutes. Mean hearing threshold was 58 dBnHL in cohorts of dogs older than 10 years, and 28 dBnHL in 5 to 10 year old dogs and dogs younger than five years. Normal ears had a mean hearing threshold of 15 dBnHL and ears with pronounced otitis externa 68 dBnHL. Results were also in line with the assessment of hearing status based on owner-provided questionnaires.<sup>13</sup> Subsequent investigations demonstrated that rapid clicks presented at 90 Hz stimulation rates binaurally could achieve similar rapid test times with comparable thresholds, further supporting previous suggestions for rapid BAER.<sup>14</sup>

Frequency-specific testing with BAER or ASSR is performed in the next step. With the aim of obtaining an audiogram over a range of frequencies reflecting human speech, usually between 0.5 and 4 kHz. BAER hearing thresholds are obtained with narrow-band tone-bursts for each frequency and predict the behavioral threshold in children. Yet BAER hearing thresholds are typically chosen by visual inspection and identification of peak V in stapled curves, and could be prone to observer bias and influenced by recording conditions.<sup>15</sup> Audiograms based on tone-burst BAER were previously recorded in dogs, but long examination times with traditional technologies and the need to perform consecutive tests for each frequency in each ear hampered their routine use in clinics.<sup>16,17</sup>

ASSR is an alternative method to obtain frequency-specific hearing thresholds. ASSR tests four frequencies simultaneously for both ears. ASSR uses narrow-band chirp stimuli, which are frequency and amplitude-modulated. Shortly, the corresponding neural response is extracted from the EEG spectrum. The audiometer provides ASSR hearing thresholds as a pass or failure for the tested frequency. Reporting is independent of observer interpretation. ASSR is considered a robust and sensitive method that can obtain an audiogram in even less time compared to next-generation BAER, with slightly lower (better) hearing

thresholds.18 Specific advantages for the use of ASSR in veterinary medicine are that the set-up and recording procedures are identical to BAER, that four frequencies may be tested simultaneously for each ear and independent from sleep-wake state. In summary, the ASSR provides an audiogram based on neural responses in uncooperative and even sedated individuals, which could offer particular advantages for veterinary patients. ASSR were introduced into veterinary medicine previously by Markessis, Poncelet et al. (2006) who concluded “that the technique appears to be a valid method of obtaining frequency specific thresholds in the canine species” and highlighted the short recording time for threshold measurement and highlighted the efficient objective computerized scoring and the high frequency-specificity.19

Details of the structured approach to newborn hearing screening are reviewed in references from human medicine: (1) initial testing includes distortion product otoacoustic emissions (DPOE) and tympanometry to assess middle ear integrity, (2) this is followed by a quick search for the overall hearing threshold with broad-band stimuli in each ear, (3) lastly frequency-specific testing with ABR or ASSR is applied in a time-efficient manner with automated detection rather than fixed number of responses and binaural test methods, testing in 20 dB and then in 10 dB steps and starting intensity 20 dB above threshold may be used. In individual cases, additional bone BAER may be performed. Bone BAER can confirm sensorineural hearing loss if absent. Alternatively, bone BAER may provide evidence for middle ear disease if air bone-gap exceeds 10 dB.20,21

### Insights from clinical patients and research applications

Hearing tests appear currently underutilized in clinics. Veterinary neurologists pursue the differential diagnosis of vestibular diseases with the neurological examination and imaging, aiming to differentiate between peripheral and central vestibular disease. While advanced imaging with high resolution may identify subtle changes, results may be normal or inconclusive in functional disorders without obvious structural changes. Hearing screening can provide further insights in these cases. Subsequent application of next-generation hearing screening in an experimental porcine animal model of genetic deafness (22) showed corresponding results for all methods, and examinations were achieved in a time-efficient manner (unpublished data). DPOAE failed frequently in older animals with dirty ear canals. ASSR appeared particularly suited for use in clinics and research due to their robustness and ease of recording. Next-generation hearing testing could be a robust supplement to the neurologic diagnostic work-up in veterinary practice.

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