ACOUSTIC CROSS-OVER BETWEEN THE EARS IN MICE (*Mus musculus*) DETERMINED USING A NOVEL ABR BASED BIO-ASSAY

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ABSTRACT

Closed-field stimulation of one ear, at high sound intensity, will activate both ears because of bone/soft tissue transmission of the acoustic signal across the skull. In human psychophysics and in clinical audiometry a knowledge of interaural attenuation values is important, particularly when assessing asymmetrical hearing loss or in studies of monaural hearing. Similarly, in testing monaural hearing in experimental animal studies, acoustic cross-over can result in erroneous conclusions about hearing function. The mouse has become a widely used animal model for various types of hearing loss, especially those relating to gene mutations, and also for age related deafness (presbycusis). In the present study we have measured acoustic cross-over in this species using a novel bio-assay technique based on auditory brainstem evoked responses (ABR). We report here for the mouse, an interaural attenuation of 37-45dB for click and 32kHz toneburst

SOMMAIRE

La stimulation de l'oreille unique à haute intensité sonore, en sphère fermée, cause l'activation des deux oreilles dù à la transmission du signal acoustique a travers les tissus mous et l'os du crâne. En psychophysique de l'homme et l'audiométrie clinique, l'atténuation interaural doit être connue dans les études d'audience mono ou de la surdité asymétrique. De même, pour tester l'audition monaurale dans les études expérimentales chez les animaux, la transmission trans-crânienne (aguillage acoustique) peut produire des conclusions erronées au sujet de la fonction auditive. La souris est devenue le modèle animal largement utilisé pour différents types de pertes auditives, en particulier celles qui ont a trait à des mutations géniques, et aussi de la surdité liée à l'âge (presbyacousie). Dans l'étude en question, nous avons mesuré la transmission trans-crânienne (aguillage acoustique) chez cette espèce en utilisant une technique de dosage biologique basée sur les potentiels évoqués auditifs (PEA). Nous rapportons ici chez la souris, une atténuation interaural de 37-45dB pour le clic et le 32kHz pip tonal.

1. INTRODUCTION

In most land vertebrates, acoustic signals in the environment reach both ears by air conduction, and differences in the time and intensity of arrival provide important cues for sound localization. In such free field stimulation, acoustic cross-over between the two ears is of little consequence. If an acoustic signal is presented directly to one ear only, i.e. closed-field stimulation, there is relatively little excitation of the contralateral ear via air conduction. At near threshold levels the activation of only one ear can be confidently assumed, however at high levels of stimulation, there is acoustic cross-over such that acoustic signals can activate both the ipsilateral and contralateral cochleas. This possibility is well appreciated in human audiological evaluations and psychophysical studies. For example in a subject with asymmetric hearing loss, high stimulation levels needed to reach threshold on the hearing-loss side may also stimulate the (lower threshold) opposite ear. This will compromise the accuracy of the audiogram. Clearly, knowledge of acoustic cross-over parameters is important (e.g. Chailkin 1967; Katz 2009). In human auditory evoked potential studies, e.g. auditory brainstem responses (ABR), noise masking of the contralateral ear can be employed to prevent evoked potential contribution from that side (e.g. Studebaker, 1967).

In experimental animal studies it can also be important to be certain that acoustic stimulation (especially if suprathreshold) is delivered to one ear alone with no acoustic cross-over. In some animal studies, experimentally induced damage to one ear is required. Evaluation of this unilateral hearing loss poses similar problems to those aforementioned in human audiology (e.g. Tonndorf, 1966).

The transmission of signal across the skull from one ear to the other has been termed acoustic cross-talk or acoustic cross-over, and is usually measured and expressed as an interaural attenuation in dB. However, the measurement of this attenuation is not straightforward,

because the transmission of an acoustic signal from one ear canal, across the skull to the opposite cochlea is complex. The primary mode of acoustic transmission is through bone conduction, but the attenuation of the signal depends much more on the soft tissue interface between the sound source and ear, and also the way in which the opposite cochlea is activated. Thus interaural attenuation can depend on sound transducer type and placement, and the spectral content of the stimulus. In various mammalian species, interaural attenuation will also vary with the bony and cartilaginous structure of the skull, the physical dimensions of the head, and the age of the animal (Tonndorf, 1966).

In the present study, we are concerned with defining acoustic cross-over in the mouse. This species has become the most used mammalian animal model for a range of biological studies. The reason for its growth in popularity is, of course, because its complete genome has been characterized, and can been manipulated to reveal various gene mutations associated with human disease. It is also a well-used animal model because it can be bred easily, and has a short life span thus useful for studies on development as well as age related pathology (including presbycusis).

In this study we employ a bio-assay in which ABR measures of auditory thresholds are recorded before and after unilateral cochlear ablation. When presenting (high level) stimuli to the ablated side, the ABR is generated from the acoustic cross-over to the normal ear. Under clinical settings, masking of the contralateral ear is carried out to prevent evoked contribution from that cochlea (Studebaker, 1967; Katz, 2002). As a control procedure, we also presented masking noise to confirm that ABRs are generated at the contralateral site. Further confirmation was made by ablating the contralateral cochlea.

2. METHODS

The animal species chosen was the CBA/J mouse (*Mus musculus*). Young male adults (6-8 weeks olds) were used. Mice were anesthetised using a Ketamine (150mg/kg) and Xylazine (10mg/kg) combination. An initial dose of 0.1mg/10gm body weight was given intraperitoneally with a half dose given every hour as needed. Mice were used in three experimental groups: a normal ABR control group (n=16), a cochlear ablation group (n=11) and a masking group (n=5). A reproducibility study (n=5) was also conducted over 7 days to ascertain the level of error made by placement of electrodes and the transducer. All procedures were approved by the local Animal Care Committee at the Hospital for Sick Children, following the guidelines of the Canadian Council on Animal Care (CCAC).

Auditory brainstem responses were recorded with electrodes placed in a vertex-to-mastoid (bulla) configuration as illustrated in figure 1. Signals were amplified (1000 X), and filtered (100-1500 Hz; Intelligent Hearing Systems, Smart-EP system). ABR measurements (512 averages) were made to 50μs clicks, and to 32kHz tone pips (2ms rise/fall times) at intensities ranging 10dB to 90dB SPL, delivered to the ear canal in a closed-field. Click stimuli were delivered using a transducer (ER2, Etymotic Research, Illinois, USA) having a spectral peak at around 8-10kHz. The 32kHz tone pips were presented with a high frequency transducer (Intelligent Hearing Systems. Miami, USA) with an effective frequency response out to 40 kHz. The mouse has a relatively high frequency range of hearing, and we have chosen acoustic stimuli in an appropriately high frequency region, i.e. a click with main spectral energy around 8-10 kHz and a 32 kHz tone pip.

In separate control studies, repeat ABR measures were made daily for 7 days to determine measurement error due to placement of electrodes and the acoustic transducer. For ABR click data, threshold measures had a standard deviation of 6.7dB. For ABR to the 32kHz stimulus, threshold measures had a standard deviation of 6.9dB. All ABR recordings were carried out in a sound attenuating room.

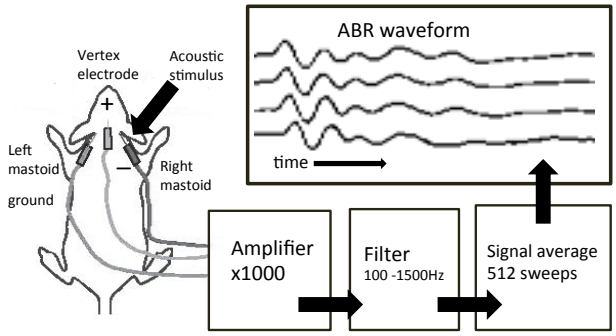

Figure 1. Electrode configuration and system diagram for ABR recording in the mouse.

Baseline ABR measurements, with acoustic stimuli delivered to each ear separately, were taken before any experimental manipulation. Left-side cochlear ablation was carried out by inserting a needle via the ear canal, through the tympanic membrane and middle ear space to pierce the cochlea. The cochlea was then flushed with water to ensure haircell damage by osmotic effects (Harrison *et al*. 1997). ABR measurements were then taken again, with sound presented to both normal and ablated ears. The mouse was allowed to recover and a second set of ABR measurements was made 24hrs later. These are referred to as post-recovery measurements. The final manipulation was ablation of the right cochlea resulting in complete bilateral hearing loss. In the masking group, a similar protocol to that outlined above

was followed but instead of a final right-side cochlear ablation, the right ear was masked using broadband noise masker at 80dB SPL.

3. RESULTS

An overview of the ABR acoustic cross-over experiment in one animal is presented in figure 2. This shows ABR waveforms and threshold levels before and after unilateral and then bilateral cochlear ablation. In this example ABRs are evoked by 32kHz tone pip stimuli.

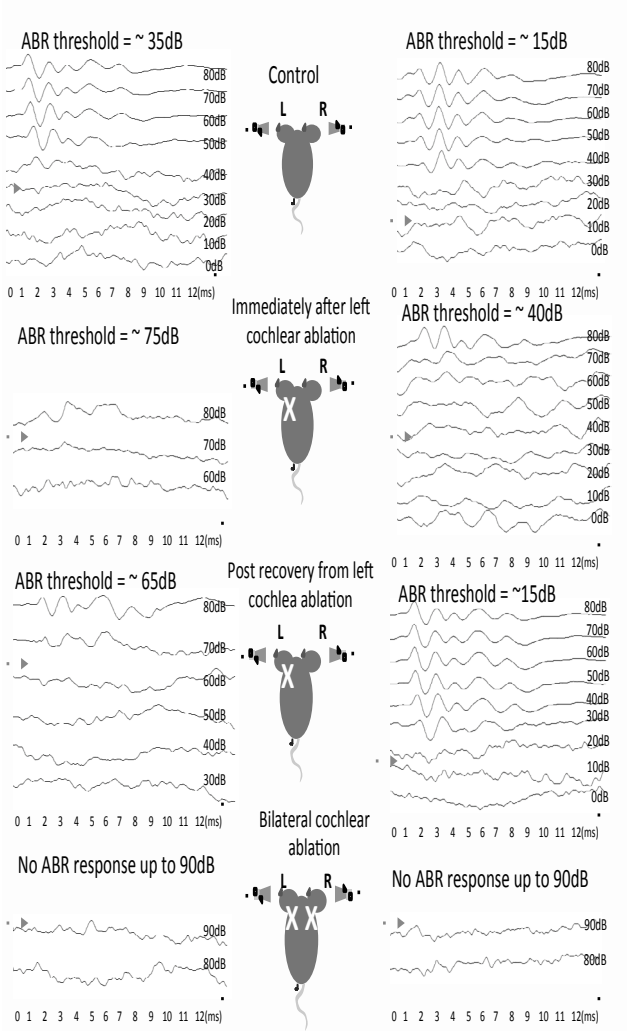

Figure 2. Schematic showing an overview of ABR measures in a mouse during an ablation study. All ABR waveforms are evoked by 32kHz tone pip. Upper panels show baseline ABR recordings to left and right ear stimulation. The approximate ABR thresholds are indicated by the arrow symbols. The next panels down show the immediate effects of left cochlear ablation on ABR waveforms, and then ABR recordings "post recovery" one day later. The lower data indicates that after both left and right cochlear ablation there are no ABR waveforms elicited.

The upper panels show the baseline ABR thresholds for stimuli in left and right ears. In this example there was some initial difference in left versus right ear thresholds. This asymmetry was sometimes found for normal mice but not typically. In any case such initial differences are not important in this study. Immediately after left cochlear ablation, ABR evoked by stimulation of the left ear shows ABR threshold elevation from ~35dB to ~75dB. Interestingly, the ABR to stimulation in the right (undamaged) ear shows a rise in threshold from ~15dB to ~40dB. This threshold returns to its original level of ~15dB a day later (post-recovery). In the left cochlea ABR threshold also drops, in this case to ~65dB at post-recovery. At this point of post recovery, differences between the thresholds in the left and right ear indicate the level of acoustic cross-over. In this example mouse it is 50dB (65dB minus 15dB).

The final manipulations post-recovery, are either noise masking or cochlear ablation of the right ear. These methods remove auditory function of the right ear and can confirm that the ABR evoked by stimulation of the left ear (after left cochlear ablation) was the result of acoustic cross-over. The subject of figure 2 had right-side cochlear ablation that resulted in a loss of any recordable ABR signal as illustrated by the lower panels. Note that for didactic reasons, ABR threshold measures given in figure 2 are approximated (+/- 5dB). In the data analysis that follows, ABR thresholds were derived more accurately using an interpolation procedure. Thus, ABR amplitudes (P2 or P3 waves) were plotted against stimulus intensity and a linear regression, extrapolated to zero amplitude provided the threshold measure.

The pooled ABR threshold data from all animals are represented in figure 3. In all subjects, ABR measures were made to click stimuli and to tone pip stimuli at 32kHz. The top graph (A) shows the ABR thresholds measured by left ear stimulation, before (filled circles), immediately after left cochlear ablation (open circles), and at post recovery (filled triangles). The effects of noise masking of the right ear (filled squares) and right ear cochlear ablation (open triangles) on ABR measures are also indicated. Panel (B) of figure 3 shows plots the ABR thresholds from stimulation of the right ear before (filled circles), immediately after left cochlear ablation (open circles), and at 24 hours post recovery (filled triangles).

With regards to the main aim of the study (the estimation of inter-aural attenuation), after left cochlear ablation all of the ABR responses to left ear stimulation originate from the right cochlear activation as a result of acoustic cross-over. Thus after ablation of the left cochlea the difference between left and right stimulation ABR thresholds is a measure of the acoustic cross-over or interaural attenuation. These values are plotted in figure 4. Because of our finding that the contralateral cochlea is influenced by the unilateral cochlear ablation, we determined interaural attenuation immediately after

ablation (black bars) and at 24hrs post-recovery (shaded bars). For the click ABR data there is a 45.3dB value for acoustic cross-over immediately post cochlear ablation, and 40.1dB after a 24 hour recovery. The difference between these values is not significant. For the 32kHz stimulus acoustic cross-over values are 41dB immediately after ablation, and 37.6dB after 24 hours, with no significant difference between values. Comparing interaural attenuation for click versus 32kHz tone stimuli we find no statistically significant difference.

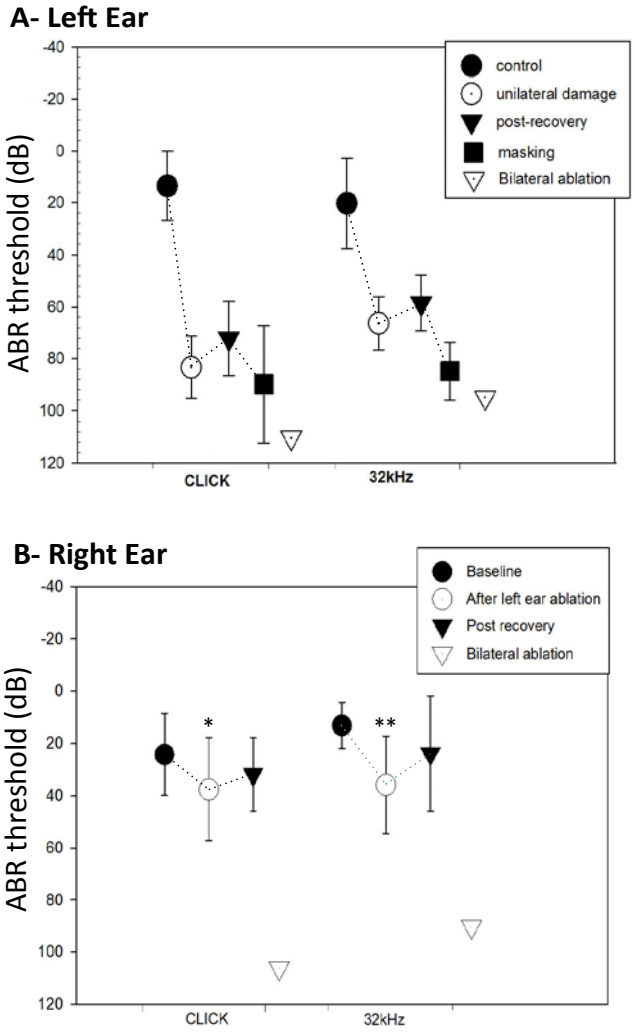

Figure 1. ABR thresholds to clicks and 32kHz tone pip stimulation presented to left ear (A) and right ear (B) before and after left cochlear ablation. Note in plot B, that immediately after ablation of the left cochlea there are significant changes in ABR threshold in the right ear (clicks, *p=0.045; 32kHz, **p <0.001).

Of interest is the finding that after left cochlear ablation, there are significant changes to ABR thresholds when stimulating the undamaged right ear. Thus as indicated in panel B of figure 3, for click and 32kHz tonal stimuli there is a statistically significant elevation in ABR thresholds immediately after cochlea ablation (for clicks, p=0.045; for 32kHz, p<0.001). This indicates that damage to the left cochlea is causing some contralateral effect on the right cochlea so as to elevate ABR thresholds by 10-20dB. We suggest that cochlear ablation results in a transient injury discharge in cochlear afferent neurons, which initiates suppression of the outer haircells in the contralateral ear via the olivo-cochlear efferent pathways (see discussion section below).

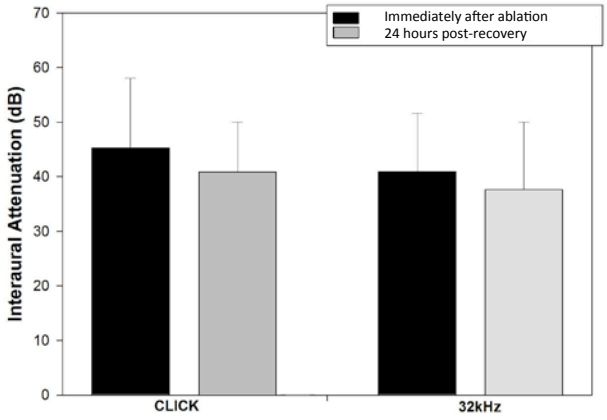

Figure 2. Interaural attenuation (acoustic cross-over) in mice (N=11) derived from ABR thresholds measured immediately after cochlear ablation (black) and at 24hrs post-recovery (light shaded).

4. DISCUSSION

Using the ABR bio-assay technique, the interaural attenuation values are 40-45 dB for click stimuli (spectral dominance at 8-10kHz) and 37-41dB for a 32kHz signal. In general these values for the mouse are lower than measurements in animal species with larger heads. Thus, in humans interaural attenuation is reported in the range of 50-85dB, depending on frequency of acoustic signal and measurement techniques (e.g. Chaiklin, 1967). In the cat, reported values range from 60-80dB (Caird *et al.*1980), in chinchilla: 50-65dB (Arnold and Burkard, 2000), and in rat: 37-75dB (Megerian *et al.* 1996). There is a general reduction in the interaural attenuation with head size that is logical. The trans-cranial signal attenuation through both bone and soft tissue elements will increase in proportion to head size, or more specifically distance between the cochleas.

There are a number of ways in which interaural attenuation can be estimated. Physical acoustic measurement can be made, for example by simply measuring the difference in level of acoustic signals in both ear canals to a unilaterally presented sound. In human subjects, behavioural measures can be employed to judge the relative intensity levels of signals reaching each ear with a unilateral signal presentation, and equivalent objective assays can be made using auditory evoked potential studies. In clinical audiology tests (both

behavioural and electrophysiological) where acoustic cross-over can be a confounding factor for estimating hearing thresholds, masking can be employed to "inactivate" one ear. In our bio-assay based on ABR measures in the mouse we can employ cochlear ablation; clearly this is not a method that can be used clinically. We suggest that this method provides a realistic and accurate estimate of functional interaural attenuation. Physical measures sample signals in the ear canal or middle ear in advance of cochlear transduction, and thus do not incorporate any auditory component. The ABR technique of the present study provides an objective and accurate measure of how an acoustic signal presented to one ear can influence the contralateral cochlea.

In the present study we have chosen to add two final "control" steps to the cochlear ablation procedure to confirm that the ABRs measured post ablation are from the contralateral side. In five mice we used noise masking (80dB SPL in the right ear) to interfere with the ability the remaining cochlea to generate a synchronized ABR. This was found to have some effect, i.e. a further 10-20dB ABR threshold elevation. The noise masking was not effective in completely obscuring an ABR signal. The definitive control, in 11 subjects, was ablation of the second ear, after which there were essentially no measurable ABRs.

Of particular interest in these data is the finding that after left cochlear ablation there are changes to threshold sensitivity in right ear. Thus in figure 3, panel B, ABR thresholds are significantly elevated by10-20dB after ablation of the contralateral (left) cochlea. One day after the ablation (post-recovery) thresholds appear to be returning to their baseline levels.

Two sources of possible inaccuracy/error need to be mentioned here. Firstly the threshold determinations for ABRs measured in separate sessions are prone to repeatability error due to slightly different electrode placements and sound source tube fit to the external meatus. We were aware of this, and as reported there were standard deviations of up to 7dB. A second source of variability in the data results from the cochlear ablation itself. The needle puncture and water irrigation of the cochlea can have different time courses of effect. We have observed that some contralateral ABR threshold changes are seen immediately, while some can take more than an hour to develop. These data are not included in the present paper because here we primarily concerned with acoustic cross-over measures, and not these contralateral neural effects. Thus in the present study we report on ABR threshold measures immediately after cochlear ablation, and in some cases the test time-window has not captured the maximal contralateral effect. Most recently we have tracked the time course of these contralateral ABR threshold changes in 12 mice and can be definitive that the ABR thresholds are significantly elevated after ablation of the contralateral

These temporary changes in ABR thresholds in the un-ablated ear are evidence that the cochleas are neurally connected. It is most likely that the ablation of the cochlea results in an injury discharge in cochlear afferent neurons. This neural discharge could result from the direct physical damage to spiral ganglion cells or from excito-toxicity caused by excessive (glutamate) neurotransmitter release from damaged inner haircells (e.g. Pujol *et al.* 1993; Olney and Sharpe, 1969). In any case the nerve will be firing as if there was a high level of acoustic stimulation to the ear. We know that such activation will cause suppression effects to outer haircells of the contralateral cochlea via the olivo-cochlear efferent pathways (e.g. Kimura and Wersall, 1962; Warr and Guinan, 1979; Liberman 1989). This phenomenon is well described in relation to contralateral suppression of otoacoustic emissions (e.g. Collet *et al.* 1990; Maison *et al.* 2000; James *et al.* 2005; Harrison *et al.* 2008).

5. SUMMARY

Using a bio-assay technique based on ABR threshold measures we have derived measures of interaural attenuation for the mouse of 37-45dB. This acoustic cross-over is relatively small compared with species with larger heads, and is for example almost half of the 50-85dB range reported for humans. Auditory researchers using a mouse model should recognize the possibility of acoustic cross-over when using monaural sound stimulation.

ACKOWLEDGEMENTS

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