Auditory Evoked Potential Recording:

Auditory evoked potential experiments were conducted in an IAC audiology booth. Animals were injected intramuscularly with an average (± S.D) of 6.4 ± 1.3 µg/g of tubocurarine hydrochloride pentahydrate (Sigma Aldrich) that had been dissolved in saline and sterile-filtered using a Millex Syringe Filter (EMD Millipore). This level reduced muscle function sufficiently to allow recordings of AEPs, while still allowing the animals to ventilate normally. When the animal was sufficiently immobilized, we applied a topical anaesthetic (2.5% lidocaine) and inserted three subcutaneous needle electrodes. The positive electrode was placed at the apex of the head, while the inverting and ground electrodes were placed in either auditory meatus directly below the tympanum. The subject was then placed in a small petri dish that was lined with moistened paper towel. We loosely draped the animal with an additional moistened towel to prevent dehydration and facilitate cutaneous respiration. To maintain a constant temperature (26 ± 1°C) the petri dish was placed on a heating pad (Snuggle Safe), which in turn was surrounded by pyramidal acoustic foam in the centre of a Faraday cage. An electrically-shielded Soundware XS satellite speaker (Boston Acoustics, U.S.A.; frequency response: 0.15-20kHz) was positioned approximately 30 cm above the subject for stimulus presentation.

Stimulus presentation and evoked potential recording were coordinated with an RZ6 processor that was connected via a fibre optic cable to a POE5 signal processing card in a computer that was running BioSigRZ (Tucker Davis Technologies, Alachua, FL). The speaker was connected directly to the RZ6. Stimuli were generated in SigGenRZ and consisted of 5-ms tone bursts with 1-ms Blackman-Harris gating. Stimulus frequencies ranged from 0.4-5 kHz in third octave steps and were presented in random order at intensities ranging from 25 to 90 dB in 5 dB steps. Stimulus presentations at lower intensities were skipped for a given frequency if a

response had not been evoked at a higher intensity. Two sets of 400 stimuli were presented in alternating phases at a rate of 21.1 stimuli per second, for each frequency-intensity combination. Evoked responses were fed from the needle electrodes to a RA4LI head stage and RA4RA preamp, which in turn fed into the RZ6 processor via a fibre optic cable. Responses were low-pass filtered at 3 kHz and digitized at a sampling rate of 24.4 kHz.

Calibration of the TDT System:

The system was calibrated by creating a 1 kHz tone of constant amplitude (1V) in SigGen and measuring the output at the speaker with a Larson Davis LxT sound level meter. The sound level of the tone was measured in a 1/3 octave band filter centered at 1 kHz. The sound level meter was set to a flat (Z) weighting. The voltage of stimuli generated in SigGen was then adjusted to match the sound level output at the speaker. This set the overall level of the system. We then calibrated the frequency response of the system using the calibration function of BioSig RZ and a Sennheiser ME62 microphone. The system creates a frequency sweep that is played through the speaker and recorded with the microphone. These responses are fed to the TDT which creates a file containing the frequency response of the speaker. From this frequency response the system creates a calibration file that automatically adjusts the voltage across frequencies to produce a flat frequency response at the speaker. The flatness of the frequency response was verified by generating tones in each 1/3 octave band and measuring the output of the speaker with a Larson Davis LxT sound level meter set to a flat response weighting and using 1/3 octave band filters. All frequencies had amplitudes that were within ± 1 dB SPL of the average tone amplitude.