Demonstration of a portable system for auditory brainstem recordings based on pure tone masking difference.

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The auditory brainstem response (ABR) has for many years been an important research tool. It has been used in many different settings from threshold determination on subjects unable to participate in ordinary psychoacoustic testing, to diagnosing tumours on the auditory nerve. The problem with most ABR systems is that they are either big, inflexible or both. Furthermore, most systems are not portable. We have developed an ABR system based on a Tucker-Davis Technologies differential amplifier and portable digital signal processor (RM2). The differential amplifier is connected via an optical cable. This system is small, weighing less than 750 grams including batteries. It is also very flexible with a graphical programming interface that makes it possible with relative ease to modify the entire system and it is relatively inexpensive (approximately 2000 USD).

We have implemented a pure tone masking difference ABR method (Berlin1991) The system has been developed for human ABR measurements and will be demonstrated at the symposium. To explore the possibilities of the system we have also used it to determine the audiograms for frogs (Rana temporaria, Xenopus tropicalis) and lizards (Gekko gecko). The audiogram is difficult to measure by behavioral methods since lizards and frogs cannot be conditioned to acoustical stimuli. ABR measurements are therefore the most convenient way to compare thresholds in the different species

**INTRODUCTION**

Non-invasive recordings of auditory evoked potentials are important in the study of human auditory function, and the auditory brainstem response (ABR) has for many years been an important research tool. It has been used in many different settings ranging from threshold determination on subjects unable to participate in ordinary psychoacoustic testing, to diagnosing tumors on the auditory nerve. Because of size and cost of the equipment it has mostly confined to clinical use in humans, but with the advent of small and relatively cheap digital signal processors with enough power to generate, record and process signals the list of possible uses of ABR in diagnostics and auditory research is expanding.

Human ABR is usually recorded by stimulating the ear with a very short click from either headphones or insert headphones and averaging the differential signal recorded
on three surface electrodes on the vertex, mastoid and zygomatic. This results in a waveform consisting of 7 peaks from I to VII. The peaks most used are I, III and V. For threshold determination, the click intensity is reduced until the response is hidden in the background noise. Peaks I and II are generated by the auditory nerve while the remaining peaks do not seem to have a single defined generator site. The maximum amplitude of a human ABR is generally less than 1 µV even under ideal conditions and often below the noise level of the recording setup.

In humans ABR is usually not the first choice for audiogram measurements, since audiometry by psychophysical methods results in more precise results faster, but when the subject cannot or will not cooperate, for instance with small children, ABR audiometry can be a useable alternative. Furthermore, ABR audiometry in connection with other physiological measurements such as otoacoustic emissions or electrocochleography can be an aid in distinguishing cochlear from later-stage hearing impairment (e.g. auditory neuropathy).

We here present a portable system for ABR measurements based on the RM2 (Tucker-Davis Technologies, Alachua, FL, USA) The RM2 is a portable DSP (digital signal processor) that is easily programmed using a graphical interface, and our tests have shown it to be a very powerful and versatile system in itself. We have already used it for sound recording, controlling experiments, student exercises and more. For ABR recordings the RM2 is connected to a preamplifier (RA4PA) and a head stage (RA4LI) via fiber optic connectors. The only serious limitation of using the RM2 is the sample rate of 25kHz, which makes it impossible to record frequencies above 12 kHz, but since the maximum relevant frequency of a ABR is usually 3kHz, it is not a problem in this case.

The problem with ordinary ABR measurements is that normally a very short click (duration less than 1ms), i.e. a broad-band sound, is used to stimulate a synchronous response in the auditory nerve, and the measurement therefore is not frequency-specific. There are different methods to make ABR measurements more frequency specific, among others the use of filtered clicks and very short tone bursts (tone pips), but the stimuli are still rather broadband signals, especially with low carrier frequencies. To avoid this problem we were using a masking method modified from Berlin et al. (1991). First, we measured the ABR using a broadband click. We then measured an ABR with the same click stimulus, but with a narrow band masker added. The masker was continuous and could therefore be narrow-band. The resulting ABR should be reduced by the masker, and the difference between the two ABRs is therefore a measure of the sensitivity to the narrow-band masker. To avoid problems of the ABR changing with time the two measurements were recorded alternately for few seconds with masker and few seconds without masker, and to avoid microphonics the phase of the signal was inverted for each presentation.

MATERIALS AND METHODS
The ABR system consisted of an RM2 digital signal processor (DSP) and a RA4PA
preamplifier both made by Tucker-Davis Technologies (TDT, Alachua, FL, USA). The preamplifier is connected with a 5 meter optical connection, and running on battery power. In some of the experiments (frogs and lizards) we were using free-field sound as a stimulus. Here, the DSP was connected to a custom made amplifier driving a loudspeaker (JBL 1G, Northridge, CA, USA). The sound levels was measured with a 1" microphone (B&K 4144, Copenhagen, Denmark) connected to a B&K 5935 Microphone supply and calibrated with a B&K 4231 acoustical calibrator. In experiments with humans, the test persons were stimulated using headphones (DENON, AH-D700, Middlesex, UK).

Frog and gecko recordings
The animal was anesthetized and mounted on a holder in the center of an anechoic chamber. All equipment was placed outside the chamber except the preamplifier and the loudspeaker. The loudspeaker was placed 1 m from the animal, on its left side. The preamplifier was placed below the animal, between the sound absorbing wedges of the floor. Responses were recorded using three subdermal needle electrode (Rochester Electro-Medical, FL, USA). One electrode (ground) was placed under the loose skin between the shoulder blades, another (zero) was placed between the eyes and the third (channel 1) was placed between the ears. The impedance was measured and the electrodes adjusted until the impedance was 3 kΩ or less between all electrodes. The hardware was controlled by custom software (QuickABR, SDU, Odense, Denmark). Each measurement consisted of the average of 400 recordings with click stimuli (UnMasked ABR) and 400 recordings where the click was masked by low intensity band limited noise (Masked ABR). The click intensity was 94dB SPL peak-peak. The masked ABR was then subtracted from the unmasked ABR resulting in a derived ABR. This method is a modified from Berlin (1991). For further analysis the derived ABR was normalized by dividing the signal by the peak-peak intensity of the unmasked ABR.

Frogs
Grass frogs (Rana temporaria) or Western clawed frogs (Xenopus tropicalis) were purchased from a commercial supplier. The animals were anesthetized by immersion in 0.3% MS222 (Ethyl 3-aminobenzoate methanesulfonate, Sigma-aldrich, St.Louis, USA) or by injection with 200 mg/kg ketamin-hydroclorid (Ketaminol Vet, 50 mg/ml, Intervet International, Boxmeer, The Netherlands ) intramuscularly in the thigh. Criteria for anesthesia was absence of response to pinching the tip of the toes. With the clawed frog the intensity of the click was 114dB SPL and each measurement consisted of 2000 recordings.

Geckos
Two young Geckos (Gecko gekko) was purchased form a commercial supplier and anesthetized by 3-4% isofluran inhalation (IsoFlo vet, Abbott Laboratories, Kent, GB) and then 150 mg/kg ketamin-hydroclorid (Ketaminol Vet, 50 mg/ml, Intervet International, Boxmeer, The Netherlands ) was injected intramuscularly in the thigh. Criteria for anesthesia was absence of response to pinching the tip of the toes. The level of
anesthesia was maintained with supplementary injections (half the initial dose) every hour. To test a common surgical procedure to prepare animals for single-cell recordings from the auditory brain stem, we tested the animals before and after the surgery. This surgery includes drilling into the skull just above the inner ear and it has been speculated that this could affect hearing threshold.

**Human**

One male human (Homo sapiens) was found by convenience sampling of the laboratory population. The subject was not anesthetized. The electrodes were Blue sensor (EKG-electrodes, Medicotest A/S, Ølstykke, Denmark) surface electrodes placed on the vertex, mastoid and zygomatic. The sound was presented by headphones and each measurement consisted of 2000 recordings. We used the same software and recording procedure as described above.

**RESULTS**

Experimental results from the grass frog and the clawed frog are shown in figure 1 and 2. The unmasked ABR of the grassfrog has an amplitude of 0.7µV and a latency of 0.5ms to peak I. In the clawed frog the unmasked ABR has an amplitude of 0.14µV and a latency of 2.5ms to peak I. The ABR in the two frog species are similar, with longer latency and lower amplitude in the less sensitive clawed frog, and the results are comparable to ABRs from the clawed frog Xenopus laevis (Katbamna 2005).

**Grass frogs**

![Grass frogs ABR graph](image)

*Fig. 1: Top trace: the unmasked ABR with an amplitude of 0.7µV and a latency of 0.5ms. Middle trace masked ABR (1400Hz, 60dB) with an amplitude of 0.6µV and a latency of 0.5ms. Bottom trace: derived response with an amplitude of 0.5µV and a latency of 0.7ms. ASNR = 12dB. The first 4ms is the delay in the system and sound travel time from the loudspeaker to the animal.*

**Clawed frog**

The frog is an aquatic species and very insensitive to sound in air. Even with a click intensity of 114 dB SPL peak-peak the ABR was very weak and with a long latency (2.5ms).
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Fig. 2: Top trace: the unmasked ABR with an amplitude of 0.14µV and a latency of 2.5ms. Middle trace masked ABR (1500Hz, 40dB) with an amplitude of 0.16µV and a latency of 2.6ms. Bottom trace: derived response with no discernible peaks. ASNR = -2dB. The first 4ms is the delay in the system and sound travel time from the loudspeaker to the animal. The animal was anesthetized by a relatively large dose of ketamine, which may have reduced its sensitivity.

Geckos

Fig. 3: Top trace: the unmasked ABR with a maximum amplitude of 6.9µV and a latency of 0.5ms for peak I and 3.2ms for peak II. Middle trace masked ABR (1500Hz, 70dB) with a amplitude of 4.0µV and a latency of 0.6ms for peak I and 2.9ms for peak II. Bottom trace: derived response with an amplitude of 4.0µV and a latency of 0.7ms for peak I and 3.3ms for peak II. ASNR = 32dB. The first 4ms is the delay in the system and sound travel time from the loudspeaker to the animal.

The results from the geckos shown in figure 3 have a maximum amplitude of 6.9µV and a latency of 0.5ms for peak I and 3.2ms for peak II. In some measurements up to three more peaks can be identified after peak II. The figure 4 shows gecko audiograms recorded before surgery, right after surgery and three days post-surgery. The pre-surgery audiogram shows a best frequency of 2 kHz with a sensitivity of 30 dB (SPL re. 20 µPa.). Right after surgery the shape of the audiogram is almost the same but the sensitivities at all frequencies is 10dB less. Three days later the audiogram is again the same shape but the sensitivity is returned to pre-surgery conditions.
Fig. 4: All the audiograms show maximal sensitivity at 2000Hz and a local maximum at 300Hz. Post-surgery thresholds are 10-20dB higher than before the surgery. After 3 days the thresholds has dropped to below the initial values. The temporary threshold shift is probably induced by drilling noise exposure during surgery.

Humans

A human ABR is shown in figure 5. The ABR has a maximum amplitude of 0.2µV and the following latencies I 1.5ms, II 2.6ms, III 3.8ms IV 4.6ms V 5.5ms VI 6.5ms. The SNR in measurements on humans was often very low for the unmasked response; this made it impossible to detect the derived response even at very high masker intensities.

Fig. 5: Top trace: the unmasked ABR with a maximum amplitude of 0.2µV and with following latencies I 1.5ms, II 2.6ms, III 3.8ms IV 4.6ms V 5.5ms VI 6.5ms. Middle trace masked ABR (2000Hz, 10dB) with same amplitude and latencies as the unmasked ABR but with less well defined peaks. Bottom trace: derived response with no discernible peaks. ASNR = 1dB. The first 1ms is the delay in the system.

DISCUSSION

A major problem determining thresholds based on ABR measurements is that the thresholds depend on the signal-to-noise ratio (SNR). The threshold is usually measured as the signal level just exceeding the level of noise, and if the SNR is high the
measured threshold will be reduced compared to lower SNRs. We are working on a normalization procedure to make thresholds independent of the SNR, but presently, we monitor the SNR and repeat the test if it is reduced.

The measurements on geckos and frogs show that the method can generate useful audiograms. These audiograms can be used to compare sensitivities of different species, to monitor animal condition during experiments and to investigate effects of anesthesia and surgery. This is especially interesting because it until now has been very difficult to determine the audiograms of lizards. The signals from frogs and lizards have high amplitude (up to 6.9 µV) and therefore a much better SNR than humans, probably because of the short distance from electrode to neural tissue. Frogs and lizards are therefore a good model system for investigations of ABR.

The measurements from humans have a low SNR, resulting from a smaller amplitude of the recorded signal (only 0.2µV). Also, the SNR in the present experiments are smaller than in a conventional ABR setup (Eclipse, Interacoustics). We are currently investigating the reason for this difference and believe that the difference reflects SNR optimization and improved signal processing in the Eclipse. However, if the SNR could be improved in the present system, it will prove a convenient, relatively inexpensive, flexible and portable system for humans as well as for other animals.

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REFERENCES
