Buenos Aires – 5 to 9 September, 2016
Acoustics for the 21st Century…

PROCEEDINGS of the 22nd International Congress on Acoustics

Psychological and Physiological Acoustics: Paper ICA2016-219

Investigation of hearing perception at ultrasound frequencies by functional magnetic resonance imaging (fMRI) and magnetoencephalography (MEG)

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Abstract

Airborne ultrasound is applied in many technical and medical processes and has increasingly moved into daily life. Because of a potential exposure of humans the question whether sound at these frequencies can be heard and whether these sounds can be of any risk for the hearing system or for wellbeing and health of an individual in general, is of great practical relevance. To study these issues audiological methods and neuroimaging were combined in order to obtain an objective rationale of the auditory perception of airborne ultrasound in humans. In a first step the monaural pure-tone hearing threshold for 26 young test subjects (19 – 33 years) in the frequency range from 14 to 24 kHz was determined. The hearing threshold values rose steeply with increasing frequency up to around 21 kHz followed by a range with smaller slope towards 24 kHz. In a next step neuroimaging techniques were applied to find brain activation following the stimulation by ultrasound between 20 and 24 kHz. Functional magnetic resonance imaging (fMRI) with sound pressure levels slightly above and below individual threshold was used in experiments with the same test persons as in the audiological measurements. Although test subjects reported audible sensation no brain activation could be identified in the above-threshold case except for the lowest test frequency at 14 kHz. Magnetoencephalography (MEG) was employed as an alternative method with the same test person group. Brain activation was measured, but again no auditory cortex activation was found above 14 kHz.

Keywords: airborne ultrasound, hearing threshold, magnetoencephalography, functional magnetic resonance imaging

1 Introduction

Airborne ultrasound is applied in many technical and medical processes and has increasingly moved into daily life. Devices using ultrasound for a variety of applications are widespread in engineering, health care and everyday live. Many of them are, collaterally or by intention, sources of airborne ultrasound. Potentially high sound pressure levels (SPL) of airborne ultrasound can for instance be produced by ultrasound cleaning or welding machines [1], exposing the worker at the machine. Animal repellents, installed in gardens, on balconies, or even in public places, produce airborne ultrasound, and many spaces of everyday life are exposed to ultrasound with non-declared and at least annoying SPL.

There are numerous indicators that airborne ultrasound events may influence human beings and that sound at frequencies above 16 kHz still can be perceived [2, 3]. However at present, the precise mechanisms of sound perception at these frequencies are hardly understood and this lack of knowledge is reflected in the status of existing – or lacking - regulations and standards. The few existing governmental guidelines for ultrasonic exposure mainly refer to the same very limited literature and knowledge base, usually assessing $^{1}/_{3}$ octave band exposure limits of about 110 dB to 115 dB for ultrasonic frequencies [4]. This situation is aggravated by an inadequate measurement infrastructure and missing metrological basis, with the result that even the determination of SPL values, a simple and common technical procedure within the audible frequency range, poses difficulties in the ultrasonic frequency range. The consequences of this unsatisfying situation are, for example, that many workplaces cannot adequately be assessed, that complaints of exposed people cannot be properly evaluated, and that manufacturers do construct noise protection housings for ultrasound machines without clear guidance, which can lead to underestimating or exaggerating the health risk.

The long term goal has to be the protection of workers and the general public from annoying or even hazardous ultrasound exposure while simultaneously protecting manufacturers and innovators from unjustified or unnecessarily restrictive regulatory measures. This can only be achieved if *all* aspects of airborne ultrasound are investigated and finally understood.

The present paper has its focus on the improvement of the understanding of the perception mechanisms of airborne ultrasound by the hearing system. For that purpose a combination of audiological methods and brain imaging was used. It was attempted to gain new insight into the physiological and cognitive mechanisms of ultrasound perception.

2 Acoustical instrumentation

Ultrasound source: To generate controlled high-frequency and ultrasonic stimuli for both audiological and objective brain measurements, a new home-made sound source was used. This ultrasound source was based on sound transmission via a tube, similar to a commercial insert earphone. The transducer used for this source was a piezoelectric loudspeaker Kemo L010 (Kemo-Electronic GmbH, Geestland, Germany) without any ferromagnetic parts. This transducer allowed the stimuli to be generated close to the ear without disturbing the imaging sensors of either an MRI or an MEG system. The loudspeaker was mounted hermetically sealed in an acoustic horn with linearly decreasing inner diameter which was coupled to the ear by a silicone tube (length: 330 mm, inner diameter: 5 mm) and by the audiometric ear tip ER3-14A (Etymotic Research, Elk Grove Village, IL), see figure 1. The coupling between the silicone tube and the ear tip was accomplished via a 3D-printed T-piece tubing (A3 in figure 1). The T-piece's middle tube was designed to closely fit an 1/8-inch pressure microphone (Brüel & Kjær 4138, without safety grid, mounted on the microphone adapter Brüel & Kjær UA0036) for calibration purposes. For easy handling, calibration and sound pressure measurements were performed at the reference plane (depicted by a dashed line in figure 1) taking into account the subsequent individual acoustic impedance, which was determined mainly by the individual ear canal geometry of the subject.

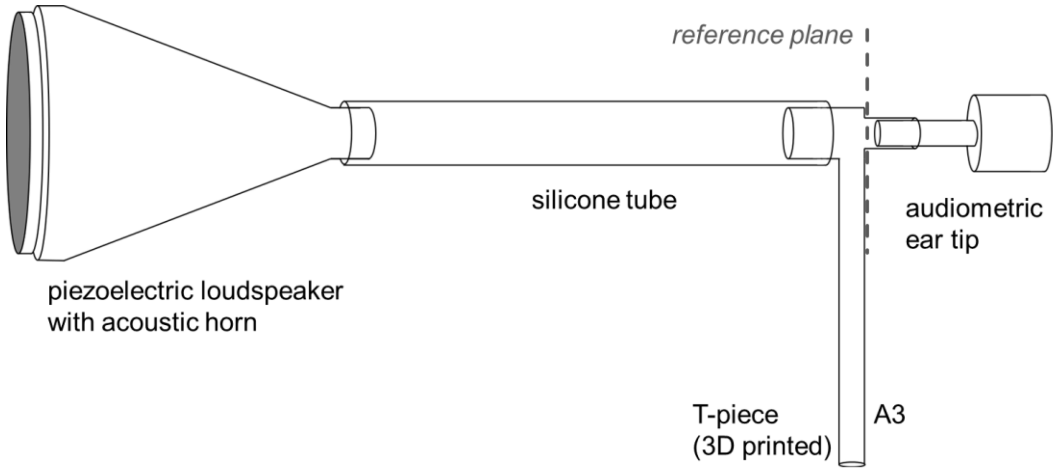

Figure 1: Schematic view of home-made insert-earphone sound source for high and ultrasonic frequencies, suitable for MEG and fMRI devices.

Stimuli: Digitally synthesized tone-bursts between 14 kHz and 24.2 kHz with a total duration of 1400 ms were used as stimuli. The bursts consisted of three ramped sinusoidal tones of 400 ms duration with a pause of 100 ms in between. On- and offset ramps were added using the hanning window function with a duration of 20 ms. In case of fMRI study, however, pure tones of 3 s duration were presented since a sparse-sampling-technique was applied.

Signals were digitally generated using a MATLAB code and converted into an analog signal by a 24 bit computer soundcard (RME Fireface UC, Audio AG, Haimhausen, Germany) using a sampling rate of 96 kHz, boosted by an amplifier (BAA 120 BEAK, BEAK electronic engineering, Frankenblick, Germany) and presented monaurally by means of the ultrasound source described above. To minimize distortions at conventional audio frequencies from the soundcard (subharmonics and intermodulation products) and to protect the test subjects from accidentally applied sounds in the more sensitive frequency range (f < 16 kHz), an active digital high-pass filter (Stanford Research Systems, Model SR 650) with a cut-off frequency f_c = 20 kHz and a 115dB/octave roll-off was used between the computer soundcard and the amplifier. In addition, a second (passive, analog) high-pass filter (f_c = 20 kHz, 12dB/octave) was installed downstream of the amplifier. It was confirmed that there were no subharmonic distortions with amplitudes above the standardized insert-earphone hearing threshold (ISO389-2:1994). Table 1 shows the selected stimulus frequencies chosen to exploit resonance enhancements in the insert-earphone sound source to achieve the necessary high sound pressure levels. To further keep residual subharmonic distortions and intermodulation products at conventional audio frequencies below the lowest hearing threshold within the subject group the maximum sound pressure level was limited individually for each stimulus frequency (table 1).

Participants: 26 test subjects (13 female and 13 male) with an age between 19 and 33 years (mean: 24.2 years) participated in the threshold-of-hearing measurements. All subjects were otologically normal which was assessed by means of the ISO 389-9 (2009) questionnaire filled out by all participants. Sub-groups of these test subjects took part in the MEG and fMRI measurements. Hence for all members of these groups individual hearing thresholds were known for a setting of stimulus levels relating to these values as a reference.

Thirteen healthy subjects with a mean age of 23.6 years (SD = 2.8) participated in the fMRI study and 9 persons took part in the MEG study. The study was conducted according to the Declaration of Helsinki, with approval of the local ethics committee. All subjects had normal or corrected-to-normal vision and normal hearing. No subject had a history of neurological, major medical, or psychiatric disorder. All subjects were right-handed as assessed by the Edinburgh handedness questionnaire. All participants took part on the basis of an informed consent.

3 Experiments

3.1 Subjective hearing thresholds

Method: All subjects received written instructions prior to the listening tests. The hearing thresholds were determined monaurally (left ear) by means of the source described in section 2. The experiment was computer controlled by the MATLAB™-based software framework “psylab”. The experimental paradigm was an unforced weighted up-down adaptive procedure as described by Kaernbach [5]. Each trial consisted of a pair of time intervals, denoted A and B, separated by a pause of 200 ms. During the acoustic presentation of these intervals the current one was indicated on a computer screen display. One of the intervals comprised the acoustic test signal, whereas the other one comprised silence. The task of the subject was to indicate, via keyboard or computer mouse, whether interval A or B contained the test signal or whether she/he was not sure. The subject had unlimited time to answer and was given visual feedback on the correctness of her/his response, whereupon the next trial began. The allocation of the test signal to the two intervals A and B was randomized for every trial. Hearing thresholds were determined in ascending frequency order beginning with 14 kHz. Measurements were aborted when the maximum sound pressure level (table 1) was reached or the “I don’t know” button was pressed subsequently 5 times, indicating that no hearing sensation existed.

Results: The resulting threshold values showed a large spread across subjects, and the number of subjects who were unable to determine a threshold at all increased with frequency. The average threshold was calculated as the median over all available individual hearing thresholds and is shown in Table 1 and Figure 2. The average threshold of hearing for a pure tone of 14 kHz was 32.9 dB re 20 µPa (dB SPL). At the highest stimulus frequency (24.2 kHz) the threshold amounts to an average sound pressure level of 110 dB SPL. It should be mentioned that only 3 out of 26 test subjects were able to perceive a tone at this frequency, while the highest possible level that the source was able to produce without audible distortion artifacts was 117 dB. The range between minimum and maximum hearing threshold values across subjects was around 50 to 70 dB for frequencies below 20.7 kHz. For higher (ultrasonic) frequencies the spreading became smaller. Despite the similar slope trend with increasing frequency, the average hearing threshold values determined in the present study are characterized by an overall offset between 8 and 20 dB in comparison to literature data (figure 3). It is assumed that the differences are caused by the use of an insert-earphone in this study instead of free-field and a different calibration process in comparison to those Ashihara [3] and Henry et al. [6] applied.

Table 1: Monaural insert-earphone threshold, maximum level of presentation, and the number of valid data (number of ears) of hearing for pure tones at f = 14 kHz and above.

f (kHz)	Max SPL (dB re 20 µPa)	Hearing threshold (dB re 20 µPa)			Number of ears
		Minimum	Median	Maximum	
14,00	108	18,1	32,9	67,1	26
15,75	111	23,5	59,3	94,8	26
16,95	115	35,4	75,1	109	26
19,10	117	59,6	98,5	114	24
20,70	118	84,1	109	117	21
21,50	113	96,5	105	109	8
22,40	113	101	106	110	8
23,75	114	109	109,5	110	4
24,20	115	109	110	111	3

Looking at the median and the minimum threshold curve in figure 3, it is obvious that the threshold increases with around 50 dB per 1/3 octave for frequencies up to 20 kHz. Above 20 kHz the slope decreases and flattens out. This decrease in slope and also the smaller spreading of data could be caused by the systematic decrease in the number of subjects who are able to hear the test tones for higher frequencies (see also table 1). Due to the limited applied sound pressure level only the most sensitive subjects (in the sense of hearing) are included in the determination of the hearing threshold for f > 20 kHz.

3.2 Magnetoencephalography (MEG) study

Procedure: The sound pressure level setting for the participants was determined individually with respect to the individual hearing threshold for each subject. MEG measurements were carried out inside a magnetically shielded room (Type Ak3b, Vacuumschmelze GmbH & Co. KG, Hanau, Germany). The signals were recorded by a commercial 128 gradiometer channel Yokogawa system. The ultrasound source was deployed inside of the Ak3b and was connected via a sound tube and an ear tip to the subjects' right ear. The left ear was closed by an ear plug.

The frequency dependent stimuli were presented in random order with an also random inter-stimulus interval between 3 and 4 seconds and a total measurement time of 40 minutes. This led to 75 epochs for averaging in MEG. The subjects had only the one duty of hearing.

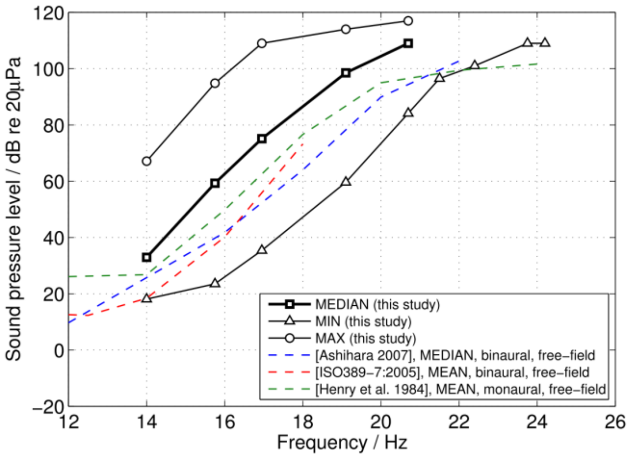

Figure 2: Monaural insert-earphone hearing threshold as median (solid squares), minimum (triangles) and maximum (circles), additionally standardized free-field hearing thresholds and data from literature are shown.

The stimuli at the frequencies 16.9 kHz, 19.1 kHz, 20.7 kHz, and 24.2 kHz were presented at two different sound pressure levels namely 2 dB below the individual hearing threshold of the subject and 5 dB above this hearing threshold. To test whether a brain response occurred at all and to observe its potential latency in the MEG plot a stimulus at a frequency of 14.0 kHz was presented at a sound pressure level of 20 dB above the individual hearing threshold. After the experiment all test persons were asked whether they had heard the ultrasound or not.

MEG data processing: The data processing of the MEG records was performed using a purpose-made code in MATLAB™ using the FieldTrip toolbox. A few non-operational channels had to be discarded and an epoch averaging was carried out on the basis of the stable trigger input. For source reconstruction a non linear dipole fit technique was applied for every stimulus to estimate the source position for every stimulus within a 3 shell BEM model consisting of the scalp, the skull and the brain tissue with a conductivity relation of 1:(1/80):1. The individual BEM models derived from T1-weighted MRI scans were matched to the Montreal Neurological Institute (MNI) template. The brain activity was modelled by two moving equivalent electric current dipoles representing the activities within the two auditory cortices. The difference between the measured magnetic signal and the calculated magnetic signal was minimised at selected time points in measurement by the Levenberg-Marquardt algorithm.

Results: None of the subjects reported a perception of the stimuli below their individual hearing threshold. The stimuli above their individual hearing threshold were recognized by 5 subjects but only 3 subjects reported to hear the 24.2 kHz stimulus. Four of the subjects only heard the 14 kHz stimulus and they were not sure about hearing sensation of the other stimuli.

All test persons showed a brain response to the reference stimulus presented at 14 kHz. As depicted in figure 3, there is an m100 (N1m) activity (brain activity around 100 ms after stimulus onset) and dipolar activation on both hemispheres was found. The latency was 117 ms and field strengths in the range of 70 fT were measured from a sensor directly above the right auditory cortex. We reconstructed two sources, one at each hemisphere at the *x*, *y* and *z* positions: 50.99 mm, 14.52 mm and 59.27 mm for the right hemisphere and -52.18 mm, 7.72 mm and 49.11 mm for the left hemisphere, respectively.

Considering this brain response as a reference for the position and the approximate field strength of the well known m100 activation, comparable signals from stimulations at ultrasound frequencies were expected. Figure 3 shows brain response signals for the stimuli above hearing threshold (left) and below hearing thresholds (right) compared to the 14 kHz response. No significant brain response could be found for stimulus frequencies of 16.9 kHz and higher and no dipole positions could be determined.

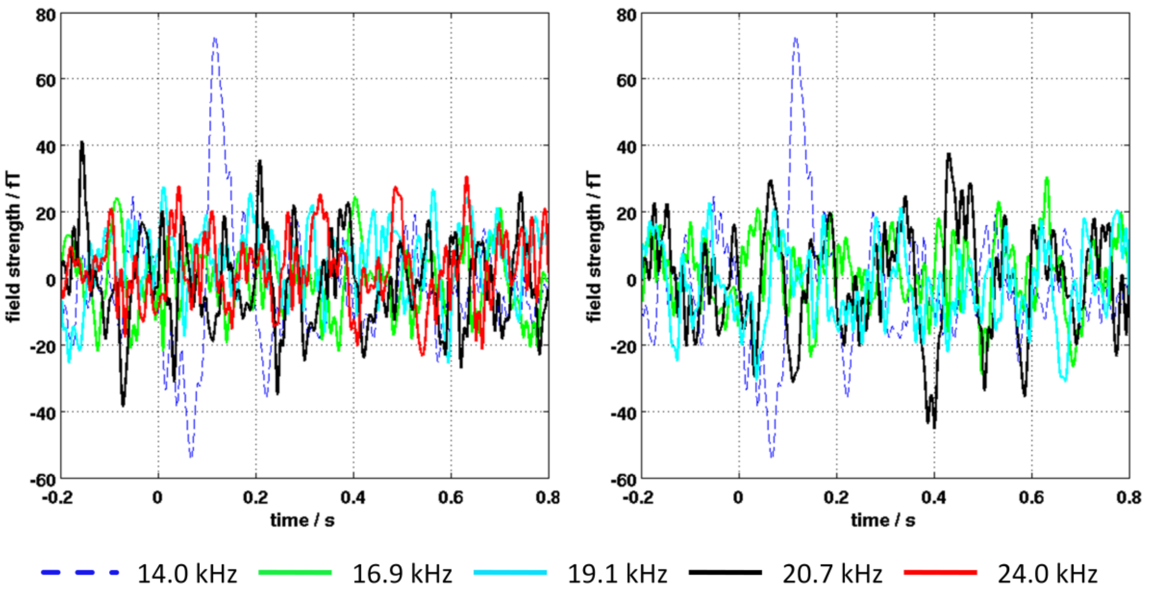

Figure 3: Acquired signals of the magnetic field from one sensor directly placed above the right auditory cortex, dashed blue line: the response to 14 kHz 20 dB above hearing threshold, left: stimuli 5 dB above threshold, right: stimuli below hearing threshold.

3.3 Functional magnetic resonance imaging (fMRI) study

Procedure: As in the case of MEG measurement the sound pressure level setting for the participants was determined individually with respect to the individual hearing threshold for each subject. In the MRI scanner participants were instructed to passively listen to the tones presented. The stimuli were pure sine tones with frequencies of 14 kHz and 24.2 kHz (each presented above the individual hearing threshold, see figure 4) as well as 16.95 kHz, 19.1 kHz and 20.7 kHz (each presented either below or above the individual hearing threshold). Each trial consisted of the presentation of one tone for the duration of 3 s. Stimulus presentations started 3 s after the sequence onset, i. e. the scanner acquired an image for 2 s then after a delay of

1 s in silence the next tone was presented. The task consisted of 280 trials, including 35 null events, where no tone was presented. All trials were distributed across 4 separate EPI-sequences. After each echo planar imaging (EPI)-sequence, participants were asked 2 questions in order to assess the subjective hearing sensation during ultrasound stimulation (1. “Did you hear the ultrasound?” 2. “Were you able to discriminate between different tones during stimulation?”). The sequence of stimuli was randomized and controlled for transition probabilities.

MRI scanning procedure: Images were collected on a 3T Verio MRI scanner system (Siemens Medical Systems, Erlangen, Germany) using a 12-channel head coil. First, high-resolution anatomical images were acquired using a three-dimensional T1-weighted magnetization prepared gradient-echo sequence (MPRAGE), repetition time 2.3 ms; echo time 3.03 ms; flip angle 9˚; 256 x 256 x 192 matrix, and 1 x 1 x 1 mm^3 voxel size. Whole brain functional images were collected on the same scanner using a T2*-weighted EPI sequence sensitive to blood oxygen level dependent (BOLD) contrast using sparse sampling (TR = 8000 ms, TA = 2000 ms, TE = 30 ms, image matrix 64 × 64 voxels, FOV = 192 mm, flip angle 80º, slice thickness 2.7 mm, 36 near-axial slices, aligned with the AC/PC line).

fMRI data pre-processing and main analysis: The fMRI data were analysed using SPM8 software (Wellcome Department of Cognitive Neurology, London, UK). The first 4 volumes of all EPI series were excluded from the analysis to allow the magnetisation to reach a dynamic equilibrium. Data processing started with slice time correction and realignment of the EPI datasets. A mean image for all EPI volumes was created, to which individual volumes were spatially realigned by means of rigid body transformations. The structural image was co-registered with the mean image of the EPI series. Then the structural image was normalised to the MNI template for the random effects analysis. The normalisation parameters were then applied to the EPI images to ensure an anatomically informed normalisation. A commonly applied filter of 8 mm FWHM (full-width at half maximum) was used. Low-frequency drifts in the time domain were removed by modelling the time series for each voxel by a set of discrete cosine functions to which a cut-off of 128 s was applied. The statistical analyses were performed using the general linear model (GLM). We modelled each trial tone frequency as a separate regressor. These vectors were convolved with a canonical haemodynamic response function (HRF) and its temporal derivatives to form regressors in a design matrix. Furthermore, six movement regressors were entered into the GLM. The parameters of the resulting general linear model were estimated and used to form contrasts. The resulting contrast image was then entered into one sample t-tests at the second (between-subject) level. We extracted beta values in the active regions and in anatomically defined region of interest (ROI) in bilateral primary auditory cortex as defined in the SPM Anatomy toolbox from each contrast between a single tone related to with the null event.

Results: In order to investigate potential auditory cortex activity in all conditions separately, we extracted beta values from the bilateral cluster and computed t-tests comparing the signal to zero. As depicted in *Figure 6* we found significant activation only for the 14 kHz stimulus and only if the latter was presented above the individual hearing threshold ($p < 0.05$). All other tone stimuli did not result in significant auditory cortex activation. These results are particularily surprising, since according to the verbal reports taken after each EPI-sequence, all 13 participants perceived the stimulation with ultrasound and were also able to discriminate different tone pitches.

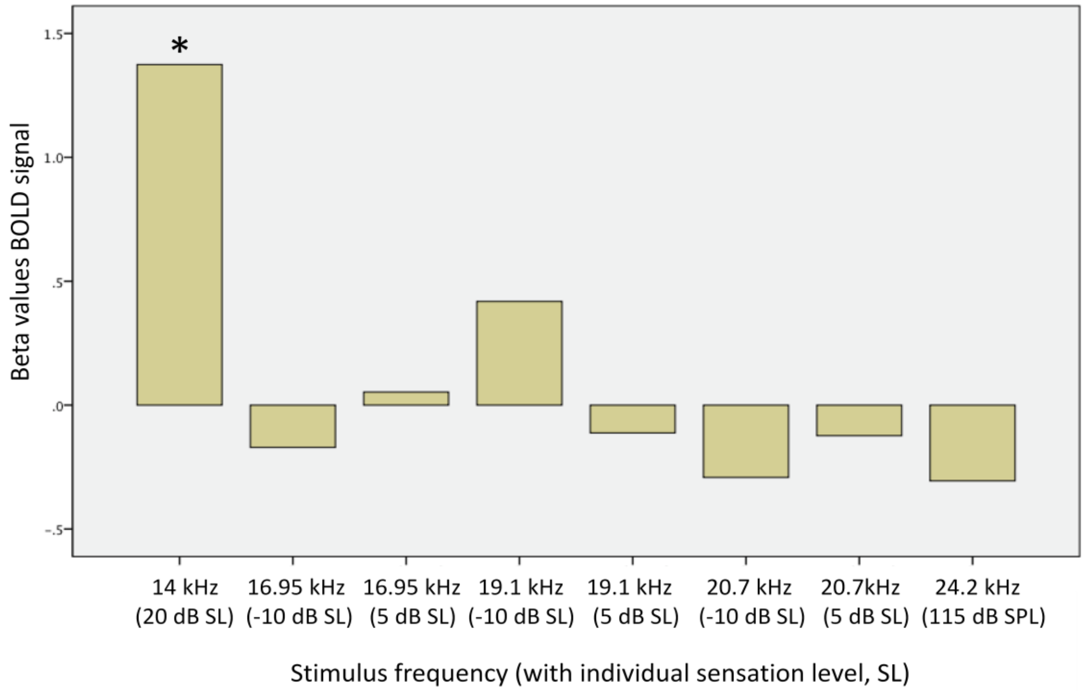

Figure 4: Beta-values of BOLD signal extracted from the primary auditory cortex during the respective tone presentation contrasted against the null event where a tone was expected, but not presented. (Asterisk: significant, SL: sensation level, 0 dB SL means the hearing threshold).

4 Discussion and conclusions

In this study the perception mechanisms of sound with high or ultrasound frequencies were investigated using audiological methods and brain imaging for an objective evaluation of the rather subjective perception. Hearing thresholds were determined up to a frequency of 24.2 kHz with a group of test persons. Later, a subgroup of these was studied using MEG and fMRI to identify brain activity in response to acoustic stimuli with a defined SPL in relation to their individual hearing threshold. No such activity indicating sound processing in the brain could be found within the brain for tones with a frequency higher than 14 kHz.

During the determination of the hearing thresholds, data could be obtained up to a frequency of 24.2 kHz. At this frequency only three of initially 26 test persons were able to hear a tone for threshold determination. Because of technical reasons, the SPL which the sound source could deliver was limited, in particular because of keeping the operation of the loudspeaker within the linear range to avoid intermodulation. The limited number of test persons depletes the quality of the threshold data at higher frequencies.

Fujioka et al. [7] could not find any brain activity in response to airborne ultrasound up to 40 kHz which is in agreement with the results of this study: No significant brain activation could be identified for frequencies higher than 14 kHz neither with MEG nor with fMRI. Although it is a serious argument that two completely different determination modalities provide the same negative result it cannot be excluded that limited sensitivity or low signal-to-noise ratio are the reasons of the missing response. Yet, one has to keep in mind that the SPLs of the ultrasonic stimuli presented during both the MEG as well as the fMRI session were set only 5 dB above the individual hearing threshold (except for our lowest test frequency of 14 kHz, see figure 5) of the test person, whereas much larger variations occurred during threshold determination. Therefore, it was not sure that during the two sessions, each supra-threshold stimulus also reliably exceeded the test person's hearing threshold.

After the threshold measurement cycles the test persons were asked to characterize their hearing sensation in case they had one. Although no quantitative measure was used almost all test persons described the hearing sensation as annoying. This allows the conclusion that at ultrasound frequencies the range of comfortable hearing is extremely small and if an ultrasound tone is heard it is immediately perceived as displeasing. The consequence for a future noise reduction or safety strategy could be to define the hearing threshold as absolute upper limit of exposure at ultrasound frequencies in order to avoid a hearing sensation at all.

The results of this study could give some guidance for the development of future safety strategies. Owing to the limited data set the results can, however, be interpreted only as a first indication that perception of airborne ultrasound by the auditory system is limited.

Acknowledgments

Financial support from the European metrology research programme (EMRP) is gratefully acknowledged. The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union.

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