

Effects of High-Intensity Airborne Ultrasound Exposure on Behavioural and Electrophysiological Measures of Auditory Function

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1 Summary

Regulations on safe ultrasound exposure limits are based on a very limited number of studies, which have only considered audiometric threshold shifts as indicators of hearing deficits. The purpose of the current study was to assess the effects of exposure to high-intensity ultrasound on a range of measures of hearing function, which included audiometric thresholds, as well as subclinical measures of hearing deficits: speech-in-noise understanding, supra-threshold auditory brainstem response wave I amplitude and latency, and frequency following response levels to amplitude modulated (AM) tones. Changes in these measures were assessed before and after exposure of the left ear to high-intensity ultrasound in a group of nine young listeners. These changes were compared to those observed in a control group of nine young listeners. Exposure consisted in the presentation of a 40-kHz AM tone at levels of 105, 110, 115, and 120 dB SPL for 10 minutes at each level, plus an exposure to a 40-kHz unmodulated tone during an ultrasound detection task, for a total duration of 50 seconds. None of the measures of hearing function was found to change significantly more for the left compared to the right ear, for participants of the exposure group compared to control participants. Electroencephalographic recordings obtained during exposure to the AM tone did not show significant phase-locked activity at the modulation frequency or at low-frequency subharmonics of the ultrasound tone. One out of nine participants was able to perform the ultrasound detection task above chance level, although due to limitations of the experimental setup the mechanism by which she could detect the presentation of the tone remains unclear.

1 Introduction

There are several sources of airborne ultrasound (US - sound with frequencies > 20 kHz) to which the general public may be exposed, such as public address voice

alarm systems, and pest deterrents [1, 2, 3]. There is also an increasing interest in the use of airborne US for the development of virtual haptic displays that can deliver tactile sensations in mid-air. These haptic displays can be used to augment the interaction with touchscreen interfaces, for example by creating virtual buttons or sliders above a touchscreen interface [4, 5]. The Ultrahaptics system consists of an array of transducers, positioned on a flat board, that generate virtual haptic displays by projecting high-intensity US at focal points in mid air; interaction of the user's unadorned hands with these focal points generates tactile sensations [4]. In order to generate tactile sensations airborne US needs to be projected on focal points on the skin at levels of around 145 dB SPL. Ambient levels at the ear will vary considerably depending on the distance and the orientation of the head of the user. With the US speakers placed at arms-length distance from the ears, the Leq obtained while rotating and translating the head across several positions has been estimated to be ~ 120 dB SPL in the absence of hand interaction with the speakers [6]. Actual user-case exposure, with hand interaction, would be expected to be lower.

There are a number of international standards and guidelines setting maximum permissible levels (MPLs) for US exposure to prevent potential adverse effects (reviewed in [7]). However, several shortcomings of the existing standards and guidelines have been pointed out recently [1, 8]. These include, but are not limited to, 1) the fact that they are almost exclusively restricted to occupational exposures, 2) the fact that they are based on sparse datasets, 3) the fact that they do not take into consideration the higher high-frequency sensitivity of some subsets of the population, such as young adults and children, who may thus not be sufficiently protected by the existing guidelines, 4) the fact that some of these standards were developed to prevent hearing threshold shifts, but not other adverse effects, such as annoyance or inability to concentrate. Moreover, there are currently no international standards for measuring US exposure in the work environment [9].

83 An additional limitation of the current guidelines
 84 for hearing protection is that they are based on studies
 85 which measured only audiometric threshold shifts as
 86 an indicator of hearing loss. In several rodent species
 87 it has been shown that noise exposure can cause a
 88 permanent loss of synapses between the inner hair
 89 cells and auditory nerve fibres. This deafferentiation
 90 of the auditory nerve can occur in the absence of a
 91 permanent threshold shift (PTS) [10, 11, 12]. This
 92 syndrome has been referred to as “cochlear synap-
 93 topathy”, and is associated in animal models with a
 94 reduction of wave I of the auditory brainstem response
 95 (ABR) at high stimulus levels, as well as with a re-
 96 duction of the frequency following response (FFR) to
 97 high-frequency (~ 1 -kHz) amplitude modulation [13].
 98 In humans, however, the results of a number of ob-
 99 servational studies have not found a clear association
 100 between noise exposure (measured with either retro-
 101 spective questionnaires or presumed on the basis of
 102 occupational status), and neural or behavioural mea-
 103 sures of cochlear synaptopathy [14, 15]. In any case,
 104 it would be desirable to check that levels of US expo-
 105 sure that do not cause audiometric threshold shifts,
 106 do not also cause subclinical hearing losses that can-
 107 not be measured by the audiogram.

108 There are different mechanisms by which airborne
 109 US could generate auditory sensations. Some of these
 110 mechanisms may operate only at certain sound fre-
 111 quencies and/or levels. At the lower range of the US
 112 spectrum, up to about 28 kHz, it is possible that
 113 US directly excites the most basal cochlear filters
 114 [16, 17]. At levels exceeding about 120 dB SPL, au-
 115 dible subharmonics of the US frequency may be gen-
 116 erated by the tympanic membrane or by the cochlea
 117 [18, 19, 20, 21, 22]. US with frequencies ranging from
 118 ~ 25 –60 kHz could also be transmitted from the eye to
 119 the inner ear via intracranial fluid conduction at lev-
 120 els as low as ~ 100 dB SPL [23]. Excessive exposure
 121 to US could therefore damage cochlear structures, in-
 122 cluding inner and outer hair cells, and the synaptic
 123 connections between the inner hair cells and auditory
 124 nerve fibres, in a way similar to low-frequency noise
 125 [24], but at the cochlear places excited by the US stim-
 126 ulation.

127 The main aim of the current study was to test the
 128 hypothesis that short exposures to US, at typical lev-
 129 els that may reach the ear of a user while interacting
 130 with the Ultrahaptics system, cause subclinical hear-
 131 ing deficits in young normal-hearing listeners. This
 132 hypothesis was tested by measuring auditory func-
 133 tion, before and after exposure of the left ear to US,
 134 with a test battery that included, besides audiomet-
 135 ric thresholds in the clinical frequency range, wave I
 136 of the ABR, the FFR to amplitude modulated (AM)
 137 tones, speech perception in noise (SPiN) thresholds,
 138 and extended high-frequency audiometry. Differen-
 139 tial left-right ear post-exposure changes in these mea-
 140 sures were compared to those of a control group of

141 participants who were not exposed to US. Two post-
 142 exposure assessments were made, one on the day im-
 143 mediately after the exposure, and one about a week
 144 after the exposure, to check for either temporary or
 145 permanent changes in the hearing measures. Addi-
 146 tionally, we attempted to measure behavioural detec-
 147 tion thresholds for the 40-kHz tone produced by the
 148 Ultrahaptics system, and analysed electroencephalo-
 149 graphic (EEG) recordings obtained during exposure
 150 to a 40-kHz AM tone, to look for traces of phase-
 151 locked neural activity at the modulation frequency,
 152 and at subharmonics of the tone. Finally, we collected
 153 subjective reports of nausea, headaches, or other pos-
 154 sible adverse subjective symptoms immediately after
 155 the US exposure.

2 Methods 156

This study was approved by the Lancaster University
 157 Faculty of Science and Technology Research Ethics
 158 Committee. The methods for this study were pre-
 159 registered on OSF: <https://osf.io/pgvdj/>. A few de-
 160 viations and additions from the pre-registered proto-
 161 col have been noted in the supplementary materials
 162 (SM). 163

A diagrammatic timeline of the experimental ses-
 164 sions is shown in Figure 1. Each session was per-
 165 formed on a different day. The average delay between
 166 the first (S1) and the second (S2) assessment session
 167 was similar between the exposure (6.33 days, $sd=1.94$)
 168 and control (5.89 days, $sd=3.52$) groups. The average
 169 delay between S2 and the third assessment session
 170 (S3) was also similar between the exposure (7.12 days,
 171 $sd=0.83$) and control (7.89 days, $sd=1.17$) groups.
 172 The delay between the US exposure (S-US) and the
 173 S2 session was, for all participants of the exposure
 174 group, of one day. 175

Each of the assessment sessions lasted about 2
 176 hours, including short breaks between the tests. The
 177 S-US session lasted about 1.5 hours. The order of the
 178 tests in the assessment sessions was always the same,
 179 starting with the measurement of audiometric thresh-
 180 olds (including the extended high-frequency region),
 181 SPiN thresholds, ABR recording, and FFR recording.
 182 The S-US session, which was attended only by par-
 183 ticipants of the exposure group, started always with
 184 the behavioural US detection test, followed by the
 185 EEG recording during US exposure. All testing took
 186 place in double-walled IAC (IAC Acoustics, Winch-
 187 ester, UK) soundproof booths. Details of all the tests
 188 will be given in the sections below. 189

2.1 Participants 190

A total of 24 native British English participants were
 191 recruited for the study from the student population at
 192 Lancaster University. An otoscopic examination was
 193 performed prior to the beginning of the tests, and six
 194

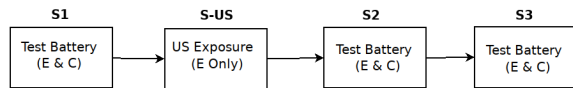


Figure 1: Timeline of the experimental sessions. The headings on top of each box indicate the session label. The text inside the box indicates the type of tests run in each session, and the group of participants tested (experimental: E; control: C). Sessions S1, S2, and S3 included the test battery for the evaluation of auditory function, and were performed by participants of both the experimental, and control group. US exposure took place during the S-US session, which was performed by participants of the experimental group only.

195 participants had to be excluded from the study due
 196 to the presence of wax occlusion in one or both ears.
 197 The remaining 18 participants (all females) were ran-
 198 domly assigned to either the exposure group ($n=9$,
 199 mean age=21 years, $sd=1.5$), or to the control group
 200 ($n=9$, mean age=21 years, $sd=1.7$). One participant
 201 of the exposure group was unable to attend the sec-
 202 ond post-exposure assessment session. Her data from
 203 the other sessions were nonetheless included in the
 204 analyses.

205 Participants were asked to limit exposure to loud
 206 noise on the day prior to each session by avoiding at-
 207 tendance to concerts or other loud venues. Although
 208 participants were not asked about previous exposure
 209 to US, given the fact that they were all students, and
 210 none spontaneously reported occupational US expo-
 211 sure, it is unlikely that their previous exposure to US
 212 would be different from that of the general popula-
 213 tion.

214 2.2 Assessment sessions - Behavioural 215 tests

216 2.2.1 Audiometry

217 Audiometric thresholds were measured for pure tones
 218 at octave frequencies from 0.125 to 8 kHz (clinical fre-
 219 quency range) as well as for pure tones at 12 and 16
 220 kHz (extended high-frequency range). The tones had
 221 a duration of 200 ms, including 10-ms cosine-raised
 222 onset and offset ramps. Thresholds were measured
 223 with a two-interval two-alternative forced-choice (2I-
 224 2AFC) paradigm. The presentation level of each tone
 225 was varied adaptively using a two-down one-up trans-
 226 formed up-down procedure tracking the 70.7% correct
 227 point on the psychometric function [25] to determine
 228 its detection threshold. On each trial the tone was
 229 randomly presented during one of two observation
 230 intervals marked by flashing lights on the computer
 231 screen, and separated by a 500-ms silent interval. Par-
 232 ticipants were asked to indicate the interval in which
 233 the sound occurred by pressing the corresponding but-

234 ton on a numeric keypad. Feedback was provided at
 235 the end of each trial by means of a coloured light on
 236 the computer screen.

237 A single block of trials was run for each combina-
 238 tion of ear and frequency (in random order). Each
 239 block was terminated after 16 turnpoints of the adap-
 240 tive track. The level was varied in 4-dB steps for the
 241 first four turnpoints, and by 2 dB for the remaining
 242 turnpoints. Threshold was estimated as the average
 243 of the last 12 turnpoints. The pure tones were synthe-
 244 sized with a sampling rate of 48 kHz, and 32-bit depth,
 245 were played through a E-MU 0204 USB sound card
 246 (E-MU Systems, Scotts Valley, U.S.A.), and presented
 247 via Sennheiser HDA300 headphones (Sennheiser elec-
 248 tronic GmbH & Co. KG, Hanover, Germany)

249 2.2.2 Speech-in-noise reception

250 Speech-in-noise understanding was assessed using the
 251 digit triplets test (DTT) [26]. On each trial the lis-
 252 tener was presented with three digits in the 1–9 range,
 253 but excluding 7 (the only digit consisting of two syl-
 254 lables). No repetitions of the same digit were allowed
 255 in a trial. The digits were voice recordings of a male
 256 speaker taken from McShefferty *et al.* [27]. A speech-
 257 shaped-noise with a root mean square (RMS) level of
 258 65 dB SPL was presented throughout the duration of
 259 the trial. The level of the speaker’s voice was varied
 260 adaptively using a one-down one-up transformed
 261 up-down procedure to determine the speech-reception
 262 threshold at the 50% correct point on the psychomet-
 263 ric function [25]. Each trial started with the recording
 264 of a female voice saying the phrase “the digits”, and
 265 was followed by the presentation of the digits spoken
 266 by the male voice. Participants were asked to input
 267 the three digits they heard, or give their best guess if
 268 they could not hear them clearly, using a numeric key-
 269 pad. Responses with repeated digits within the same
 270 sequence were not allowed. Feedback was provided at
 271 the end of each trial by means of a coloured light on
 272 the computer screen.

273 A block of trials was terminated after 16 turnpoints.
 274 The target level was changed in 2-dB steps for the first
 275 four turnpoints, and by 1 dB for the remaining turn-
 276 points. Threshold estimates for each block of trials
 277 were based on the average of the last 12 turnpoints.
 278 Participants completed two blocks of trials for each
 279 ear (first one block for each ear, in random order; then
 280 a second block for each ear, in random order). The
 281 participants’ thresholds were estimated as the average
 282 of the threshold estimates obtained in each of the two
 283 blocks of trials for each ear. The recordings of the
 284 digits had a 48-kHz sampling rate and 16-bit depth.
 285 They were digitally mixed with the speech shaped
 286 noise, played through a E-MU 0204 USB sound card,
 287 and presented via Sennheiser HD650 headphones.

2.3 Assessment sessions - EEG tests

For these tests the EEG was recorded with the Biosemi ActiveTwo system (BioSemi B.V., Amsterdam, The Netherlands). Gold-plated active electrodes were used. One electrode was attached on the middle of the forehead, just below the hairline, one on the neck, at the level of the 7th cervical vertebrae, and one on each earlobe. The common mode sense and driven right leg electrodes were attached on the forehead. The EEG signal was acquired at a sampling rate of 16.384 kHz with 24-bit resolution. Stimuli were generated with a sampling rate of 48 kHz and 32-bit resolution, were played through a 24-bit RME Hammerfall DSP multiface DAC (RME Intelligent Audio Solutions, Germany), and presented via mu-metal shielded ER3A Etymotic insert earphones (Etymotic Research Inc., Elk Grove, U.S.A.). Triggers marking the start of a stimulus were sent to the Biosemi receiver from additional channels of the soundcard after being transformed to discrete pulses by a custom-built device.

2.3.1 Auditory brainstem response

The ABR was recorded in response to 100- μ s, 100-dB ppeSPL clicks in rarefaction polarity. The clicks were presented at a rate of 14.1 per second, with alternate presentation between the left and right ear. A total of 10,000 clicks were presented (5,000 to each ear).

The EEG was bandpass filtered offline between 0.1 and 1.5 kHz [28] with a 256-taps zero-phase-shift finite impulse response (FIR) filter. The triggers marking click onsets were adjusted to compensate for the 0.9-ms delay introduced by the earphones tubing, and the EEG was then segmented into discrete epochs relative to the onset of the clicks using a -2 to 12 ms time window. The forehead channel was re-referenced to the ipsilateral earlobe channel. All the analyses were performed using this montage. The segments were baseline corrected by subtracting the mean amplitude during the 2-ms pre-stimulus window, and averaged using the iterative weighted averaging algorithm [29]. The ABR wave I peaks were identified using an automatic peak-picking procedure which is described in the SM. Log-transformed peak amplitudes [30, 31] were used in the statistical analyses.

2.4 Frequency following response

The FFR was recorded in response to two simultaneous AM tones with carrier frequencies of 0.59 and 2 kHz, and modulation frequencies of 93.3, and 124.4 Hz, respectively. Each tone was presented at a level of 75 dB SPL. The tones were embedded in pink noise to reduce the contribution of high-spontaneous rate fibres to the recorded FFRs. The pink noise was presented at a spectrum level of 40 dB SPL *re.* 100 Hz, in a frequency region from 20 to 3000 Hz, with

notches two equivalent rectangular bandwidths [32] wide around the carrier frequencies so as to form three noise bands (20–506, 683–1773, and 2253–3000 Hz). The stimuli had a duration of 450 ms, including 10-ms onset and offset raised-cosine ramps. Two-thousand stimuli were generated (1,000 for each ear; half with the tones in condensation, and half with the tones in rarefaction polarity), each with a fresh noise sample, and saved on disk. FFRs were collected in a single block of trials with the 2,000 stimuli presented in a random order. The inter-stimulus interval was jittered between 25 and 75 ms.

The EEG was bandpass filtered offline between 0.06 and 1 kHz with a 256-taps zero-phase-shift FIR filter. The triggers marking stimulus onsets were adjusted to compensate for the 0.9-ms delay introduced by the earphones tubing, and the EEG was then segmented into discrete epochs relative to the onset of the stimuli using a -5 to 450 ms time window. The forehead channel was re-referenced to the neck channel. All the analyses were performed using this vertical montage. The segments were baseline corrected by subtracting the mean amplitude during the 5-ms pre-stimulus window, and averaged using the iterative weighted averaging algorithm [29].

Spectral analyses were used to determine the level, in dB, of the FFR at each modulation frequency. The waveforms were windowed using a hamming window, and the waveform spectra were computed via fast Fourier transforms (FFTs). The signal level was estimated by the power at the FFT bin closest to the signal frequency.

2.5 Ultrasound Tests

US tones were presented using the Ultrahaptics array as a loudspeaker source pointing straight towards the left ear from a position to the left, and slightly to the front (angle \sim 25 degrees), of the participant, at a distance of \sim 112 cm from the left ear.

There are particular challenges associated with measuring SPL at ultrasonic frequencies. Complex field patterns are formed meaning deviations in microphone position of a few centimetres can have extreme effects [33]. Moreover, the complex interaction of the sound field with the head, torso and pinna mean that free-field measurements do not provide a full and meaningful picture of individual exposure levels [34]. For these reasons we adopted a calibration procedure that would provide meaningful output with the potential to be replicated by other researchers in different labs. Moreover, in the interest of safety, any deviation from accepted free-field measurements should result in an overestimate of SPL.

The level of the US tones was calibrated with a Brüel & Kjær (Nærum, Denmark) type 4191 microphone fitted in the ear of a Brüel & Kjær type 4100 head and torso simulator (HATS). The microphone

398 grid formed a flush boundary at the entrance to the
 399 ear canal of the HATS pinna. Therefore all US SPLs
 400 presented in the article are estimates of SPLs at the
 401 eardrum of the participant. The HATS was positioned
 402 on the chair where the participants would be perform-
 403 ing the tests. The positions of the chair, and of the
 404 US speaker arrays were fixed throughout the experi-
 405 ment (they were the same during the calibration pro-
 406 cedure and when participants were tested). Micro-
 407 phone data were acquired through a Picoscope (Dr-
 408 DAQ, Pico Technology) which was programmed for
 409 real-time SPL measurement with a 1/3 octave band
 410 filter centred at 40 kHz. A digital equalization filter
 411 was implemented to convert the free-field response of
 412 the microphone to a pressure-field response. Thus, all
 413 SPLs represent an estimate of actual incident pressure
 414 at the interface. SPL values were exponentially time
 415 weighted using a 1s time constant; equivalent to the
 416 'SLOW' setting on a standard SPL meter.

417 Various 40-kHz US tones were presented, varying
 418 the signal voltage in order to find the voltage values
 419 that would result in SPLs of 100, 105, 110, 115, and
 420 120 dB. This procedure was repeated with the HATS
 421 placed in three slightly different orientations: looking
 422 straight ahead, with the head slightly tilted towards
 423 the right, and straight ahead with the torso propped
 424 up by about 15 cm. The measurements for the first
 425 position (looking straight ahead) were repeated twice
 426 after repositioning the HATS. Each of these four
 427 datasets was fitted with a function to estimate the re-
 428 lation between voltage and output level. The RMS er-
 429 ror between the recorded SPLs and the ones predicted
 430 by the estimated functions across the four function fits
 431 was 1.7 dB. The difference in the SPLs predicted by
 432 the function fits for the two datasets obtained with the
 433 HATS looking straight ahead was 2.44 dB. The maxi-
 434 mum difference in the SPLs predicted by the function
 435 fits across the four datasets was 4.77 dB. These data
 436 indicate that slight changes to the position of the head
 437 of the participants would result in level changes of
 438 around 5 dB, or less. Because we intended to present
 439 US tones close to the MPLs set by the International
 440 Labour Office [35] we chose to calibrate on the fits
 441 obtained with the HATS position that predicted the
 442 highest SPLs (head slightly tilted towards the right),
 443 so that deviations in the position of the head from
 444 this reference position would result in slightly lower
 445 SPLs rather than in higher SPLs.

446 During all of the US tests, the right ear of the par-
 447 ticipant was plugged with a 3M E-A-R classic soft
 448 foam earplug (3M Company, Maplewood, U.S.A.),
 449 so that only the left ear would be exposed to high-
 450 intensity US. Tests conducted at Ultrahaptics indicate
 451 that, properly fitted, these earplugs provide about 30
 452 dB of attenuation at 40 kHz. To investigate the possi-
 453 bility that US exposure could elicit adverse subjective
 454 effects, at the end of the US tests participants were
 455 presented with the following written question: "Have

456 you experienced dizziness, loss of balance, feeling sick,
 457 headaches, or a feeling of pressure/fullness in the ears
 458 during the test? If yes, please specify which symptoms
 459 you have experienced".

2.5.1 Behavioural ultrasound detection 460

461 The ability to detect a 500-ms 40-kHz tone was as-
 462 sessed using a 2I-2AFC paradigm. On each trial the
 463 tone was randomly presented during one of two obser-
 464 vation intervals marked by flashing lights on the com-
 465 puter screen, and separated by a 500-ms silent inter-
 466 val. Participants were asked to indicate the interval in
 467 which the tone occurred by pressing the corresponding
 468 button on a numeric keypad. Feedback was provided
 469 at the end of each trial by means of a coloured light
 470 on the computer screen. A hybrid adaptive/constant
 471 procedure [36, 37] was used: The presentation level of
 472 the tone was initially varied adaptively using a two-
 473 down one-up transformed up-down procedure to de-
 474 termine its detection threshold. However, the presen-
 475 tation level was limited to a maximum of 120 dB SPL,
 476 if the adaptive track reached this level at any time
 477 (including the initial turnpoints) the adaptive track
 478 was terminated early, and the procedure switched to
 479 a constant one to estimate the proportion of correct
 480 responses at the maximum level of 120 dB SPL until
 481 a total of 50 trials at this level had been completed.
 482 Otherwise the block was terminated after 16 turn-
 483 points. The tone level was initially set at 110 dB, and
 484 was changed in 4-dB steps for the first four turnpoints,
 485 and by 2 dB for the remaining turnpoints. If the track
 486 converged, the threshold was estimated as the average
 487 of the last 12 turnpoints. Each participant completed
 488 two blocks of trials.

489 The electronic board of the US speakers generated
 490 a noise below the ultrasound frequency range when
 491 the speakers were playing. This noise was clearly au-
 492 dible, and its level increased when the output level
 493 of the US tone increased. In an attempt to prevent
 494 listeners from responding to this noise rather than to
 495 the US tone, a 34-sec sample of the noise generated by
 496 the speakers was recorded with a Zoom Q3HD (Zoom,
 497 Tokyo, Japan) portable recorder and played back to
 498 mask the noise generated by the US speakers. The
 499 masking noise was lowpass filtered at 16 kHz, and
 500 100, 2.5-sec samples drawn at random starting points
 501 from the 34 sec recording were extracted. The spec-
 502 tra of the noise recording and the masker are shown in
 503 Figure S1 in the SM. The masker samples were played
 504 back through two JBL 305P MkII speakers (JBL Pro-
 505 fessional, Northridge, U.S.A.) symmetrically placed
 506 around the US speakers, at a level at the listener's left
 507 ear of 71 dB C-weighted during each trial. The mask-
 508 ing noise started 0.5 seconds before the start of each
 509 trial, ended 0.5 seconds after the end of each trial, and
 510 was gated on and off with 50-ms raised-cosine onset
 511 and offset ramps.

The level of the masking noise was established by preliminary tests during which the first author (SC) ran several blocks of the US detection task varying the masker level across blocks in 10-dB steps to find the level at which he was performing the task at chance level. The level of the masker for the main experiment was set 30 dB above the level at which SC was performing at chance level. SC had normal hearing in the clinical frequency range up to 8 kHz for both ears at the time of the tests. No data are available on SC's hearing sensitivity above 8 kHz, but it should be noted that, given that he was 37 years old at the time of the tests, it is unlikely that he would have been able to hear not only the 40-kHz tone, but also its first subharmonic.

2.5.2 EEG recordings during ultrasound presentation

The EEG was acquired in response to a 40-kHz US tone amplitude modulated at a rate of 124.4 Hz. Four blocks of trials, in which the level of the tone was either 105, 110, 115, or 120 dB SPL were run. It was not possible to send a trigger with sub-millisecond accuracy from the US speaker array to the EEG system. For this reason, during each block a single US tone was presented continuously for 10 minutes. The four blocks were randomly ordered. The EEG acquisition settings and electrode configurations used for this test were the same as the ones used for the EEG tests in the assessment sessions, and described in Section 2.3.

The FFR is largest for tones with frequencies around 500 Hz, and can only be recorded for tones with frequencies below about 2,000 Hz [38]. For this reason we limited the analysis to subharmonics 6 to 8 of the 40-kHz carrier (corresponding to frequencies of 625, 312.5, and 156.25 Hz), as well as to the modulation frequency of 124.4 Hz. The EEG was bandpass filtered offline between 60 and 1,000 Hz with a 256-taps zero-phase-shift FIR filter. The forehead channel was re-referenced to the neck channel. All the analyses were performed using this vertical montage.

To improve the signal-to-noise ratio (SNR) the continuous recordings were split into shorter segments which were then averaged. To ensure that the phase of the signal of interest was coherent across segments, the 10-min recording was split into consecutive segments. Four different segmentations were performed with segment durations of 1, 2, 4, or 5 seconds, so that an integer number of cycles would fit into a segment for signal frequencies of 625, 312.5, 156.25, and 124.4 Hz, respectively. For each segmentation the segments were then separately averaged. The resulting waveforms were windowed using a hamming window, and the waveform spectra were computed via FFTs. For each of the target signal frequencies the level of the signal and of the noise were estimated from the FFT obtained from the corresponding segmentation procedure.

The signal level was estimated by the power at the FFT bin closest to the signal frequency. The noise level was estimated by summing the power of 1-Hz bands above and below the signal bin, but excluding a 2-Hz band above and below the signal frequency to minimize the effects of spectral leakage on the noise estimate.

2.6 Statistical analyses

Statistical analyses were run in R [39] by means of Welch two-sample *t*-tests. The tests were specified in the pre-registered protocol. There are four main families of tests corresponding to the research questions described in the introduction:

- Does US exposure (at the levels, frequencies, and durations used in this study) have any *temporary* effects on hearing function as assessed by behavioural and psychophysical measures?
- Does US exposure (at the levels, frequencies, and durations used in this study) have any *permanent* effects on hearing function as assessed by behavioural and psychophysical measures?
- Are 40-kHz US tones detectable behaviourally?
- Are 40-kHz AM US tones detectable from FFR recordings?

For the first research question, for each measure of interest we first computed the difference between values obtained for the left and the right ears, then the difference of the resulting values between S2 and S1. These between-session changes of between-ear differences were the dependent variables that were compared between the exposure and control groups by means of *t* tests. The measures of interest were:

- Average audiometric threshold across the clinical audiometric range (0.125–8 kHz; $PTA_{0.125-8}$).
- Average audiometric threshold across the extended high-frequency range (12 and 16 kHz; PTA_{12-16}).
- SPiN threshold.
- Log-transformed wave I ABR amplitude.
- Wave I ABR latency.
- FFR level at the modulation frequency for the 0.59-kHz carrier ($FFR_{0.59}$).
- FFR level at the modulation frequency for the 2-kHz carrier (FFR_2).

We thus performed a total of seven tests, and set the α level to $0.05/7 \simeq 0.007$ using a Bonferroni correction for multiple comparisons, and one-tailed tests because in each case the hypothesis was directional. For

simplicity, for these, and all other tests, uncorrected p values will be reported in the paper; significance should be assessed with respect to the specified α level.

The second research question is analogous to the first one, but involves differences between S3 and S1 (instead of differences between S2 and S1) to test for permanent changes in auditory function. For this research question we thus also performed a total of seven one-tailed t tests, and set the α level to $0.05/7 \simeq 0.007$.

For the third research question, previous studies [16, 17] have shown that there are large interindividual variations in the detectability of ultrasound even within the population of young normal hearing listeners. For some listeners detection thresholds have been measured up to a frequency of 28 kHz, while for other listeners thresholds are already unmeasurable at a frequency of 20 kHz. For this reason we did not run group-level statistical analyses. Instead the detectability of the tone was assessed separately for each listener. Given that this required a total of nine tests across listeners, the α level was set at $0.05/9$. Plans on how to assess if detectability was above chance in case one or both blocks of the hybrid adaptive/constant procedure converged to a threshold were given in the pre-registration protocol. However, for none of the listeners did any of the blocks converge to a threshold, so we will only consider the case in which the proportion of correct responses at 120 dB SPL is available for both blocks of trials. Following binomial probability, a listener should get at least 63 correct out of 100 responses to provide evidence of detection at greater than chance level at an α level of $0.05/9$.

For the fourth research question, it is also likely that there may be large interindividual differences, hence we did not run group-level statistical analyses. The presence of a signal for each participant, level, and frequency tested can be detected using an $F_{2,2m}$ test [40] where m is the number of bins used to compute the noise power. This test is based on the fact that FFT power estimates have a χ^2 distribution both at the signal frequency, and at neighbouring frequencies. Therefore their ratio can be tested using an F statistic. Because power at the signal frequency is the sum of two independent squared variables (the real and imaginary parts) the signal power estimate is distributed as a χ^2 variable with 2 degrees of freedom, while the noise power estimate, which is obtained by averaging m bins, is a χ^2 variable with $2m$ degrees of freedom. Running a test at each of the four levels, for each of the four target frequencies, and for each of the nine participants required a total of 144 tests, so the α level was set at $0.05/144$. The number of noise bins falling in the two 1-Hz bands above and below the target bin varied according to the segment duration, and was 2, 4, 8, or 10 bins, respectively, for segment durations of 1, 2, 4, or 5 seconds. Following the equations in [40] the criterion SNR to detect a significant

signal at the α level of $0.05/144$ was therefore set at 20.18, 13.86, 11.02, and 10.48 dB for the 625, 312.5, 156.25, and 124.4 Hz signals, respectively.

3 Results

3.1 Audiometry

The audiograms for each participant, session, and ear are shown in Figure S2 in the SM. The average audiograms for each combination of ear, session, and group were close to 0 dB HL, although thresholds tended to be slightly higher at 16 kHz.

Overall the audiograms across S1 and S2 appeared relatively stable for both the control (SM Figure S3), and the exposure (SM Figure S4) group. A few listeners from either group showed apparent losses or gains of sensitivity > 10 dB at one or more frequencies. However, in these cases the standard deviation of the turnpoints of the adaptive track of the block of trials with the highest threshold was almost invariably high, suggesting that these changes were due to a high lapse rate in that block of trials. One listener from the exposure group showed an apparent threshold shift of more than 30 dB at 1 kHz for the exposed ear. However, this listener did not show large threshold shifts for the exposed ear at the other test frequencies. Out of concern for the participant her 1-kHz thresholds were immediately re-tested twice for both the left, and the right ear. The participant was asked to pay full attention to the task before re-testing. Her 1-kHz threshold for the left ear went down from 34 to -20 dB HL in the first repetition, and then to -10 dB HL in the second repetition. Her 1-kHz thresholds for the right ear were quite stable across the three repetitions, around -4.5 dB HL. Given that her thresholds went back to normal in the re-tests it is clear that the apparent threshold shift was a false alarm most likely caused by a high lapse rate. Nonetheless, to avoid bias the data from the two re-tests were not used further. Only the original data with the threshold shift have been analysed and used for the figures and statistical tests reported in the manuscript.

Figure 2 shows, for each group, the difference in average audiometric thresholds across the clinical, and the extended high frequency ranges, between the left and right ear, between session 2 and session 1. A loss of sensitivity for the exposure group in the left (exposed) ear relative to the right ear would manifest as an increase in the threshold difference shown in the figure. Averaged across participants, the threshold differences were small, less than 1.1 dB in absolute value, for both groups in either frequency range. The t -tests comparing the threshold differences between the exposure and the control group did not reveal significant differences either in the clinical range ($t_{(11.987)} = 0.944$, $p = 0.182$), or in the extended high frequency range ($t_{(15.57)} = -0.331$, $p = 0.628$).

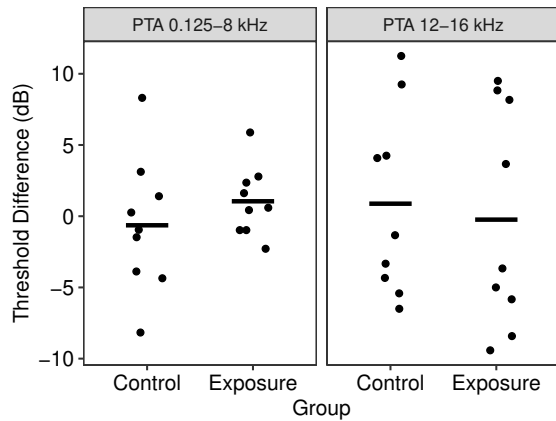


Figure 2: Difference in audiometric thresholds between the left and right ear between session 2 and session 1: $(T_{L2} - T_{L1}) - (T_{R2} - T_{R1})$, where T refers to the threshold, the first subscript indicates the ear, and the second subscript the session number. An *increase* in the threshold difference in the exposure group would indicate a relative post-exposure loss of sensitivity in the left (exposed) ear compared to the right ear. Points plot individual listeners' data. Segments plot group averages.

729 The difference in average audiometric thresholds
 730 across the clinical and the extended high frequency
 731 ranges, between the left and right ear, between ses-
 732 sion 3 and session 1, are shown for each group in Fig-
 733 ure S5. Threshold differences were small, less than
 734 1.5 dB in absolute value, for both groups in either
 735 frequency range. The t -tests comparing the thresh-
 736 old differences between the exposure, and the control
 737 group did not reveal significant differences either in
 738 the clinical range ($t_{(12.247)} = 0.85$, $p = 0.206$), or in
 739 the extended high frequency range ($t_{(13.761)} = 0.248$,
 740 $p = 0.404$).

741 3.2 Speech in noise reception

742 The DTT thresholds for each participant, session, and
 743 ear are shown in Figure S6 in the SM. DTT thresh-
 744 olds were relatively stable across sessions for both the
 745 exposure and the control group. Figure 3 shows, for
 746 each group, the difference in DTT thresholds between
 747 the left and right ear, between session 2 and session 1.
 748 A decrement in SPiN for the exposure group in the left
 749 (exposed) ear relative to the right ear would manifest
 750 as an increase in the threshold difference shown in the
 751 figure. Average threshold differences were small, less
 752 than 0.5 dB in absolute value, for both groups. The t -
 753 test comparing the threshold differences between the
 754 exposure and the control group did not reveal a sig-
 755 nificant difference ($t_{(15.529)} = -0.283$, $p = 0.609$).

756 The difference in DTT thresholds between the left
 757 and right ear, between session 3 and session 1, are
 758 shown for each group in Figure S7. Threshold differ-

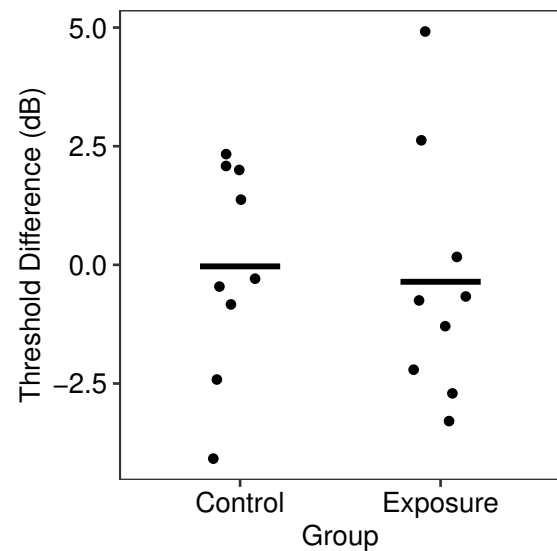


Figure 3: Difference in DTT thresholds between the left and right ear between session 2 and session 1: $(T_{L2} - T_{L1}) - (T_{R2} - T_{R1})$, where T refers to the threshold, the first subscript indicates the ear, and the second subscript the session number. An *increase* in the threshold difference in the exposure group would indicate a relative post-exposure performance drop for the left (exposed) ear compared to the right ear.

ences were small, less than 1 dB in absolute value, for
 both groups. The t -test comparing the threshold differ-
 ences between the exposure and the control group
 did not reveal a significant difference ($t_{(13.799)} =$
 -0.693 , $p = 0.75$).

764 3.3 Auditory brainstem response

765 3.3.1 Wave I ABR amplitude

766 Figures S8 and S9 in the SM show the ABR waveforms
 767 for each participant of the control and exposure group,
 768 respectively. ABR grand averages for each group are
 769 shown in Figure 4. The wave I ABR amplitudes for
 770 each participant, session, and ear are shown in Fig-
 771 ure S10 in the SM. ABR amplitudes were remarkably
 772 stable across sessions for both the exposure, and the
 773 control group. Figure 5 shows, for each group, the
 774 average geometric ratio in wave I amplitude between
 775 the right and left ear, between session 2 and session
 776 1. A decrement in wave I amplitude for the exposure
 777 group in the left (exposed) ear relative to the right
 778 ear would manifest as an increase in the amplitude
 779 ratio shown in the figure. Average amplitude ratios
 780 were close to one, with average amplitudes changing
 781 by less than 10% in either direction, for both groups.
 782 The t -test comparing the log-transformed amplitude
 783 (log-amplitude) differences between the exposure, and
 784 the control group did not reveal a significant difference
 785 ($t_{(15.887)} = -1.315$, $p = 0.896$).

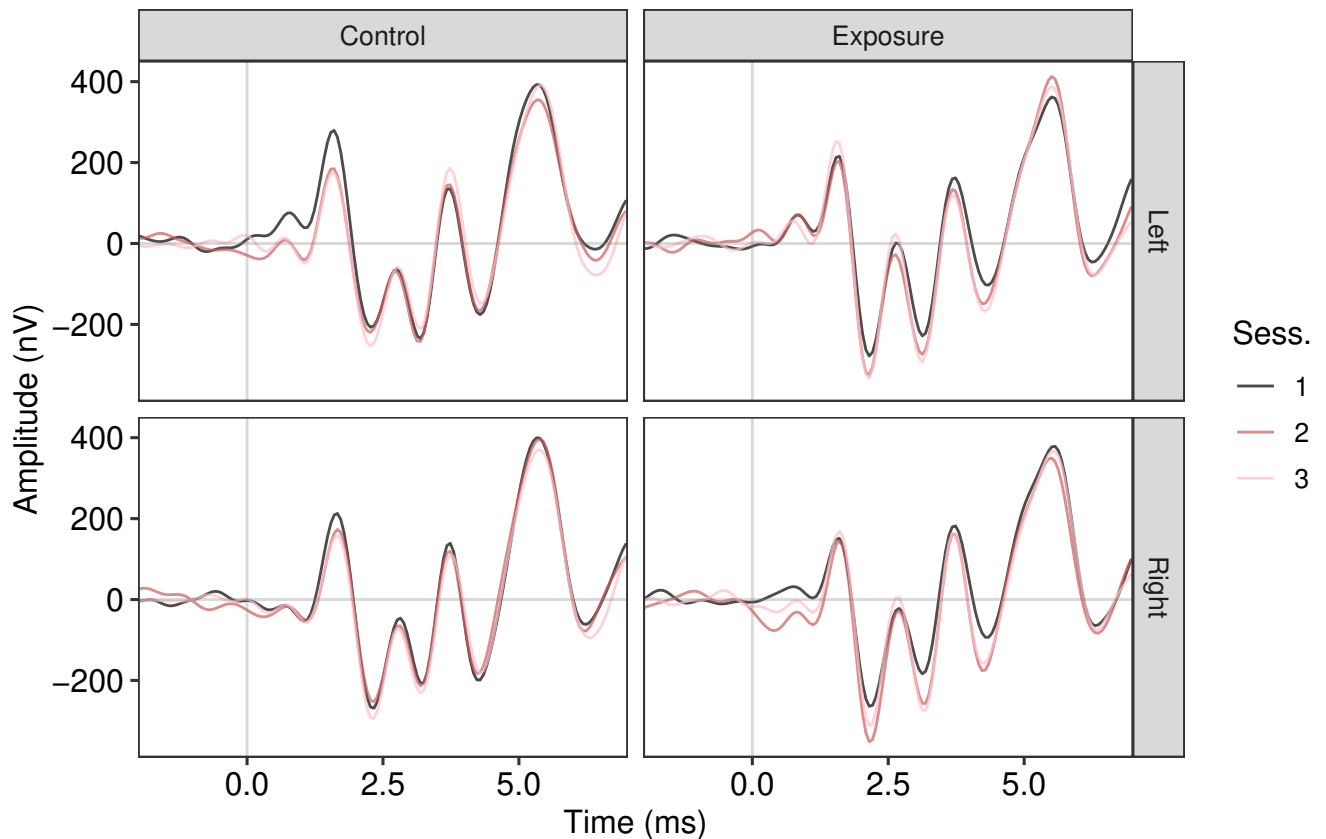


Figure 4: (Colour online) ABR grand averages.

786 Geometric average wave I amplitude ratios between
 787 the right and left ear, between session 3 and session
 788 1 are shown in Figure S11. Average amplitude ratios
 789 were close to one. The t -test comparing the log-
 790 amplitude differences between the exposure, and the
 791 control group did not reveal a significant difference
 792 ($t_{(11.165)} = -1.982, p = 0.964$).

793 3.3.2 Wave I ABR Latency

794 The wave I ABR latencies for each participant, ses-
 795 sion, and ear are shown in Figure S12 in the SM. ABR
 796 latencies were quite stable across sessions for both the
 797 exposure, and the control group. Figure 6 shows, for
 798 each group, the average difference in wave I latency
 799 between the left and right ear, between session 2 and
 800 session 1. An increase in wave I latency for the ex-
 801 posure group in the left (exposed) ear relative to the
 802 right ear would manifest as an increase in the latency
 803 difference shown in the figure. Average latency differ-
 804 ences were close to zero for both groups. The t -test
 805 comparing the latency differences between the expo-
 806 sure, and the control group did not reveal a significant
 807 difference ($t_{(15.436)} = 1.201, p = 0.124$).

808 Average wave I latency differences between the left
 809 and right ear, between session 3 and session 1 are
 810 shown in Figure S13. Average latency differences were
 811 close to zero for both groups. The t -test comparing

812 the latency differences between the exposure, and the
 813 control group did not reveal a significant difference
 814 ($t_{(11.804)} = 0.321, p = 0.377$).

815 3.4 Frequency following response

816 The FFR levels for each participant, session, ear, and
 817 carrier frequency are shown in Figure S14 in the SM.
 818 FFR levels were less stable across sessions than ABR
 819 amplitudes for participants of both groups. Figure 7
 820 shows, for each group and carrier frequency, the av-
 821 erage differences in FFR levels between the right and
 822 left ear, between session 2 and session 1. A decre-
 823 ment in FFR level for the exposure group in the left
 824 (exposed) ear relative to the right ear would manifest
 825 as an increase in the level difference shown in the fig-
 826 ure. Average FFR level differences were in the range
 827 of a few dBs, but the variability was large. The t -
 828 tests comparing the threshold differences between the
 829 exposure and the control group did not reveal a sig-
 830 nificant difference either at the low ($t_{(13.844)} = 1.208$,
 831 $p = 0.124$), or at the high ($t_{(15.885)} = -0.535, p = 0.7$)
 832 carrier frequency.

833 Average differences in FFR levels between the right
 834 and left ear, between session 3 and session 1 are
 835 shown in Figure S15. Average FFR level differences
 836 were in the range of a few dBs, but the variability was

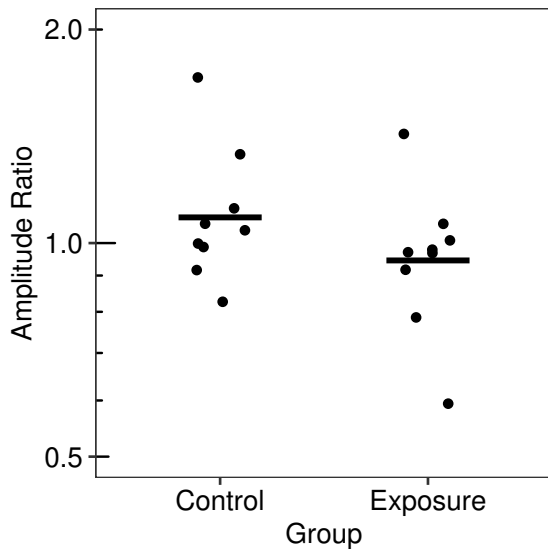


Figure 5: ABR wave I amplitude ratio between the right and left ear, between session 2 and session 1: $(A_{R2}/A_{R1})/(A_{L2}/A_{L1})$, where A refers to the amplitude, the first subscript indicates the ear, and the second subscript the session number. An *increase* in the amplitude ratio in the exposure group would indicate a relative post-exposure wave I amplitude decrease in the left (exposed) ear compared to the right ear.

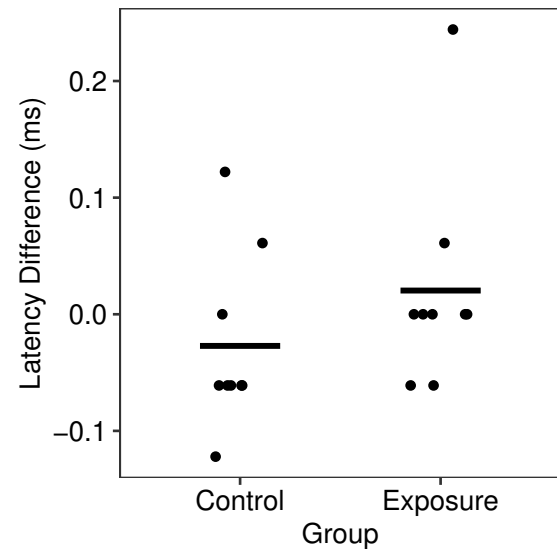


Figure 6: ABR wave I latency difference between the left and right ear, between session 2 and session 1: $(T_{L2} - T_{L1}) - (T_{R2} - T_{R1})$, where T refers to the latency, the first subscript indicates the ear, and the second subscript the session number. An *increase* in the latency difference in the exposure group would indicate a relative post-exposure wave I latency increase in the left (exposed) ear compared to the right ear.

837 large. The t -tests comparing the threshold differ-
 838 ences between the exposure and the control group
 839 did not reveal a significant difference either at the
 840 low ($t_{(12.844)} = 1.208$, $p = 0.124$), or at the high
 841 ($t_{(14.371)} = 0.612$, $p = 0.275$) carrier frequency.

842 3.5 Confidence intervals

843 Although the lack of significant differences in the de-
 844 pendent variables measured in this study does not
 845 provide evidence of either temporary or permanent ef-
 846 fects of US exposure on hearing function, they should
 847 not be taken on their own as evidence against this
 848 hypothesis. It is useful to look at interval estimates
 849 to understand the range of possible effects that the
 850 results of the experiment could support. Confidence
 851 intervals (CIs) do not necessarily reflect measure-
 852 ment precision, and cannot be generally interpreted as
 853 Bayesian credibility intervals covering the $X\%$ most
 854 probable values of a parameter of interest [41], al-
 855 though under some assumptions, for simple normal
 856 models CIs and credibility intervals are often quite
 857 similar [42, 43]. For this reason, besides computing
 858 CIs, we also computed Bayesian credibility intervals.
 859 Credibility intervals were computed as 99% highest
 860 density intervals (HDIs) of the posterior distribution
 861 of the parameter of interest [44]. Posterior distribu-
 862 tions were obtained by means of Markov Chain Monte
 863 Carlo sampling using JAGS [45] and R [39]. The
 864 JAGS model code is provided in the SM. The depen-

865 dent variables (between-session changes of between-
 866 ear differences were) were modeled with a normal like-
 867 lihood function and heterogeneous variances between
 868 groups. Priors were vague on the scale of the data.
 869 CIs and HDIs for all the tests involving differences be-
 870 tween S1 and S2 are shown in Table 1. CIs and HDIs
 871 for all the tests involving differences between S1 and
 872 S3 are shown in Table S1 in the SM. To be consistent
 873 with the one-tailed tests performed in this study, the
 874 CIs need to be one sided, and corrected for multiple
 875 comparisons. These are provided in the first column
 876 of the tables. However, one-sided CIs are unbound
 877 on one side; two-sided CIs provide a more intuitive
 878 understanding of the uncertainty of the parameters of
 879 interest. The second column of the tables provides
 880 99% CIs uncorrected for multiple comparisons (note
 881 that an uncorrected two-sided 99% CI is practically
 882 quite close to a two-sided 95% CI corrected for seven
 883 multiple comparisons using the Bonferroni method).
 884 The third column of the tables provides 99% HDIs.

885 3.6 Behavioural ultrasound detection

886 For all of the participants the adaptive track reached
 887 a level of 120 dB in both blocks of trials, hence the
 888 procedure switched in each case to a constant one es-
 889 timating the proportion of correct responses at 120
 890 dB SPL. This proportion is shown for each partici-
 891 pant in Figure S16 of the SM. For eight of the nine
 892 participants performance in the task was at chance

Variable	Corrected 95% CI (one-tailed)	Uncorrected 99% CI (two-tailed)	Bayesian HDI
PTA _{0.125-8}	-3.42-Inf	-3.77-7.14	-4.76-8.11
PTA ₁₂₋₁₆	-10.44-Inf	-11.03-8.79	-13.21-11.07
DTT	-3.49-Inf	-3.69-3.04	-4.44-3.75
ABR WI Log-Amp.	-0.43-Inf	-0.45-0.17	-0.52-0.23
ABR WI Lat.	-0.06-Inf	-0.07-0.16	-0.09-0.19
FFR _{0.59}	-6.41-Inf	-7.14-16.85	-9.49-19.43
FFR ₂	-8.79-Inf	-9.25-6.39	-11.06-8

Table 1: Interval estimates for the changes between S1 and S2 for the dependent measures analyzed in the study. The first column shows 95% one-sided CIs corrected for multiple comparisons. The second column shows uncorrected 99% CIs. The third column shows 99% Bayesian HDIs.

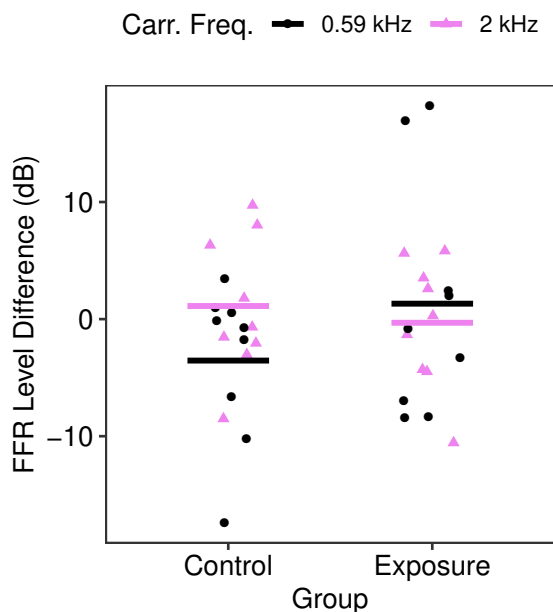


Figure 7: (Colour online) Difference in FFR level between the right and left ear, between session 2 and session 1 ($M_{R2} - M_{R1} - (M_{L2} - M_{L1})$), where M refers to the level, the first subscript indicates the ear, and the second subscript the session number. An *increase* in the level difference in the exposure group would indicate a relative post-exposure decrease in FFR level for the left (exposed) ear compared to the right ear.

level. However, one participant performed clearly above chance level, with 94 out of 100 correct responses.

3.7 EEG recordings to ultrasound

The FFR SNR at the target subharmonic frequencies, and at the modulation frequency of the 40-kHz AM tone for each participant of the exposure group is shown in Figure S17. For none of the participants did the FFR SNR reach the criterion for statistical significance in any condition. Figure S18 shows the across-participant average SNR for each condition. The av-

erage SNR was in each case close to zero.

3.8 Subjective effects

Seven participants reported no subjective effects or symptoms after the US exposure session. Two participants reported generic effects likely unrelated to US exposure. The first one (P17) reported feeling “a bit fidgety” during the session, but told the experimenter that this was probably related to having to sit still for the entire duration of the session. The second one (P23) reported a “slight feeling of pressure/fullness in ears – initially when earplugs inserted and then more towards the end”. It should be noted that towards the end of the session this participant was exposed to the lowest US levels (105, and 110 dB SPL).

4 Discussion

In this study we assessed the performance of a group of young listeners on a series of behavioural and electrophysiological hearing tests before and after their left ear was exposed to high-intensity US. Their performance changes were compared to those of a control group of listeners who were not exposed to US. Additionally, participants of the exposure group performed behavioural, and electrophysiological tests to assess the detectability of the US to which they were exposed. The results can be summarized as follows:

- We did not find evidence that US exposure, at the levels, frequencies, and durations used in the current study has any temporary, or permanent effects on hearing function as assessed by several psychophysical, and electrophysiological measures.
- Only one out of nine listeners was able to detect the presentation of a 40-kHz 120 dB SPL US tone. Due to limitations of the experimental setup, however, it is unclear whether this listener was able to hear the tone itself, one of its subharmonics, or extraneous level/spatial cues asso-

941 ciated with the low-frequency noise made by the
942 US speakers when they were playing US.

- 943 • We did not find evidence that low-frequency sub-
944 harmonics (< 1 kHz), or the modulation fre-
945 quency, of an AM 40-kHz US tone presented at
946 levels ranging from 105 to 120 dB SPL could be
947 detected electrophysiologically using the FFR.

948 A discussion of each of these points will be presented
949 in the following sections.

950 4.1 Effects of ultrasound exposure

951 We did not find evidence of either temporary, or per-
952 manent audiometric threshold shifts as a result of ex-
953 posure to US, which included the presentation of 40-
954 kHz US tones at levels of 105, 110, 115, and 120 dB
955 SPL for 10 minutes at each level. The 99% HDIs
956 for the threshold difference in the clinical and ex-
957 tended high frequency range suggest that, even if US
958 exposure at the levels and durations used in the cur-
959 rent study would lead to a temporary threshold shift
960 (TTS), the shift could not be larger than about 11 dB.
961 We are aware of only three published studies, reviewed
962 by Lawton [46], that have investigated the presence of
963 temporary or permanent threshold shifts after expo-
964 sure to US at similar, or higher levels than those used
965 in the current study. Parrack [47] found that 5-min
966 exposures to US tones between 21 and 37 kHz at lev-
967 els ranging from 148 to 154 dB SPL caused TTSs at
968 subharmonics of the US frequencies; these TTSs sub-
969 sided rapidly and did not lead to PTSs. Grigor'Eva
970 [48] failed to find TTSs after one-hour long exposures
971 to a 20-kHz US tone of either 110, or 115 dB SPL.
972 The reports of these two studies do not provide the
973 number of participants tested, nor demographic infor-
974 mation. Acton and Carson [49] measured the audio-
975 grams of 16 workers before and after a working day
976 which involved exposure to various drills and washers
977 that produced sounds with one-third octave band lev-
978 els sometimes in excess of 100 dB SPL at ultrasonic
979 frequencies, and below about 90 dB SPL at lower fre-
980 quencies. Although they found a few large TTSs at
981 individual frequencies for some of the ears tested (6%
982 of the datapoints), because of their random pattern,
983 and the fact that some of the shifts were positive and
984 some were negative, the authors attributed the shifts
985 to measurement variability and did not attach any
986 particular significance to them. No detailed informa-
987 tion on the age of the participants tested is provided,
988 except for the fact that most of the men tested had
989 some degree of presbycusis and were older than the
990 women. In an additional study Di Battista [6] did
991 not find evidence of TTSs in a group of 10 partici-
992 pants ranging in age from 24 to 64 years, after 5-min
993 exposures to 40-kHz US tones ranging in level from
994 100 to 120 dB SPL. Overall, the results of our study
995 are consistent with those of these previous investiga-

tions that did not find significant audiometric TTSs
after exposure to US up to levels of 120 dB SPL.

In addition to the lack of significant changes in au-
diometric thresholds, we did not find evidence of ef-
fects of US exposure on subclinical measures of hear-
ing function that included DTT thresholds, wave I
amplitude and latency measurements, and the level
of the FFR to AM tones. Average DTT threshold
changes were close to zero, but caution should be exer-
cised in interpreting this result because the 99% HDI
for DTT threshold differences is compatible with the
possibility of threshold increases after US exposure
of up to 3.75 dB. For comparison, the average differ-
ence in DTT thresholds between normal hearing and
hearing-impaired listeners is about 4 dB [50]. In any
case, given that the most important frequency region
for speech perception lies below ~ 5 kHz, and that
TTSs have been found at most for the third subhar-
monic of a US tone [47], it seems unlikely that DTT
thresholds could be affected as a result of exposure
to a 40-kHz US tone. The lack of significant effects
of US on the ABR and FFR is potentially more in-
formative, because at high stimulus levels large sec-
tions of the cochlea contribute to these responses, and
both are greatly affected by the contribution of basal
(high-frequency) cochlear sites [51, 52]. The 99% HDI
for wave I ABR amplitude suggests that our results
would be compatible with potential relatively small
wave I log-amplitude reductions of at most 0.23, which
corresponds a decrease in amplitude of $\sim 20\%$. For
comparison, wave I amplitude reductions as a func-
tion of age in a 40-years span have been estimated to
be around 38%, after accounting for concomitant re-
ductions due to hearing loss in the 2–4 kHz frequency
range [53]. The 99% HDI for ABR wave I latency sug-
gests that our results could be compatible with mod-
est latency changes of at most 0.19 ms. For compari-
son, wave I latency increases as a function of age in a
40-years span have been estimated to be around 0.25
ms, after accounting for concomitant latency changes
due to hearing loss in the 2–4 kHz frequency range
[53]. Due to the relatively high variability of FFR
levels obtained in this study the 99% HDIs for poten-
tial US-exposure related reductions in FFR level are
large, and compatible with changes of up to 19.4 dB
for the 0.59-kHz carrier, and of up to 8 dB for the
2-kHz carrier.

One limitation of the current study is that the pre-
vious history of US exposure of the participants was
not known. If potential negative effects of US do
not increase linearly with the historical amount of
exposure but plateau after a certain threshold, and
the exposure history of our participants had reached
this threshold, any negative effects would have been
missed in our study. Because our participants were
recruited from the student population and did not
spontaneously report a history of occupational US ex-
posure, it is unlikely that their exposure would be

different from that of the general public. However, quantifying non-occupational US exposure would be very challenging given the increasing number of US sources in public places [1, 2, 3], and given that these US sources are generally inaudible.

4.2 Behavioural detection of ultrasound

Eight listeners were unable to detect a 500-ms 40-kHz tone, presented at a level of 120 dB SPL, but one listener (P14) performed the 2I-2AFC task clearly above chance level with 94% correct responses. Previous studies [16, 17] have shown that some listeners were able to detect US tones up to a frequency of 28 kHz, while none of the listeners tested were able to hear US tones of 30 kHz. A pink noise was used in these previous studies to ensure that participants could not perform the detection task by listening to subharmonics of the US tones. The maximum presentation level in these studies was 110 dB SPL. The presentation level of the US tone used in our study was 10 dB higher. Given that the frequency of our tone was more than 10 kHz higher than the highest detectable frequency in previous studies, it seems unlikely that the higher SPL used in our study would have been sufficient to make a 40 kHz tone detectable, although we cannot rule out this possibility. Two alternative possibilities remain to explain the results of P14. The first one is that for this listener the masker was not sufficiently intense to mask the low-frequency noise produced by the speakers, or the spatial cues arising from the different positions of the US speaker array, and the speakers playing the masker. However, given that the level of the masker was set 30 dB above the level at which the first author, who is highly experienced in psychoacoustics tasks, was performing at chance level, this possibility seems somewhat unlikely. The second possibility is that this listener was able to perform the task by detecting the first subharmonic of the US tone, which would have fallen at a frequency of 20 kHz. Although no 20-kHz component is visible in the spectrum of the recording of the US tone (see Figure S1), at high SPLs subharmonics have been detected in physiological recordings from non-human animals [20]. These subharmonics are thought to be generated mainly by the tympanic membrane in the middle ear, although some may be also generated by the cochlea [21]. Although in humans, subharmonics radiated from the eardrum have only been recorded at levels of at least 140 dB SPL [18], theoretical models predict that levels of ~ 120 dB SPL could be sufficient to generate them [19, 22]. Given that the masker used in the current experiment was lowpass filtered at 16 kHz, a subharmonic at 20 kHz would not have been masked and may have been detectable by the listener who performed the detection task above chance level. Her 16-kHz threshold for the left ear, averaged across

sessions, was -2.7 dB HL, the second best, and one of the only three <10 dB HL among participants of the exposure group. Hence, this listener would have been more sensitive to the presence of a 20-kHz subharmonic than most other listeners of the exposure group.

4.3 Electrophysiological detection of ultrasound

A number of studies have investigated the effects of ultrasonic stimulation on neurophysiological responses in humans using EEG, magnetoencephalography or neuroimaging techniques. The results have been mixed; some studies have failed to detect cortical activity evoked by US stimuli [54], while other studies, comparing stimuli with and without ultrasonic components, have found differences in the power of certain EEG frequency bands or detected a greater activation of some brain regions in response to stimuli with ultrasonic components using neuroimaging methods [55, 56]. Our study differs from the previous ones because we investigated the detectability of US using the FFR, a steady-state evoked potential response that, if present, contains energy at frequencies harmonically related to those of the stimulus, or generated by non-linear interactions in the auditory system [38, 57].

Because the FFR can only be detected for frequencies below ~ 2 kHz, and for stimuli ~ 40 – 45 dB above perceptual threshold [38], we had a priori low expectations of finding FFRs to the AM US tone employed in this experiment. TTSs have been detected only up the third subharmonic of a US tone, and at levels much higher than those used in the current study. Thus it was unlikely that subharmonics of a 40-kHz tone could be detected in the frequency region below 2 kHz where the FFR can be recorded. Although the 124.4 Hz modulation frequency falls into this frequency region, given that the highest frequency at which US has been detected (while subharmonics were masked) is 28 kHz [16, 17], it is unlikely that even the most basal cochlear filters could be responding to the 40-kHz AM tone components to generate a response at the modulation frequency. Acoustic recordings of the AM US tone showed the presence of a component at the modulation frequency of 124.4 Hz, probably generated by modulation distortions in the air [58]. Although it was not possible to establish the level of this component, its level was likely too low to be detected via the FFR. Overall, the absence of FFRs to the US tone found in our study is not surprising.

4.4 Subjective effects

Only two participants reported minor subjective effects after US exposure, but these were vague and possibly unrelated to US presentation. Sensitivity to US may be limited to a sensitive subset of the population,

1164 and various research reports, reviewed by Leighton
 1165 [1], indicate that only some people manifest negative
 1166 symptoms when they are nearby US sources. In our
 1167 study we did not specifically recruit participants with
 1168 a history of negative reactions to US sources, and
 1169 given that our sample size was small it is possible
 1170 that none of our participants belonged to a subset of
 1171 the population who may have a heightened sensitiv-
 1172 ity to US. Adverse reactions to the presence of a US
 1173 source may be partly psychogenic, and it is unclear to
 1174 what extent interindividual differences in reactions to
 1175 US reflect actual differences in hearing sensitivity or
 1176 psychological differences [59]. It is possible that both
 1177 play a role depending on the specific frequencies and
 1178 levels of the US components, that in turn determine
 1179 their audibility.

1180 All the participants of the exposure group had nor-
 1181 mal hearing for the exposed ear up to 12 kHz, and
 1182 only two of them had thresholds slightly above 20 dB
 1183 HL for the exposed ear at 16 kHz. For this reason
 1184 we can exclude that the lack of major reactions to US
 1185 in our study was due to poor high-frequency hearing.
 1186 Given the high interindividual variability of thresh-
 1187 olds for sounds in the ultrasonic frequency range even
 1188 for young normal hearing listeners [16, 17, 54], it is
 1189 nonetheless possible that our sample did not include
 1190 enough participants with sufficient sensitivity to ob-
 1191 serve major negative reactions to US exposure. In-
 1192 deed only one of our participants was able to detect
 1193 the presentation of the US tone, but this participant
 1194 did not show any negative subjective reactions.

1195 4.5 Conclusions

1196 We did not find evidence of either audiometric thresh-
 1197 old shifts or changes of behavioural or electrophysio-
 1198 logical subclinical measures of hearing function in a
 1199 group of young participants exposed to US up to lev-
 1200 els of 120 dB SPL, compared to a control group. Our
 1201 results are consistent with previous studies that did
 1202 not find audiometric threshold shifts after exposure to
 1203 US at similar levels. Our sample size was relatively
 1204 small, consisting of nine participants per group, and
 1205 caution should be exercised in interpreting the null
 1206 results. However, analyses of the credibility intervals
 1207 for the dependent measures suggest that any effects if
 1208 they existed, would not be large, with the exception
 1209 of the FFR measures, which were quite variable and
 1210 did not yield precise estimates.

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 1219 The underlying data in this paper is available from
 1220 <https://osf.io/pgvdj/>.

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Supplementary figures referenced in the main manuscript

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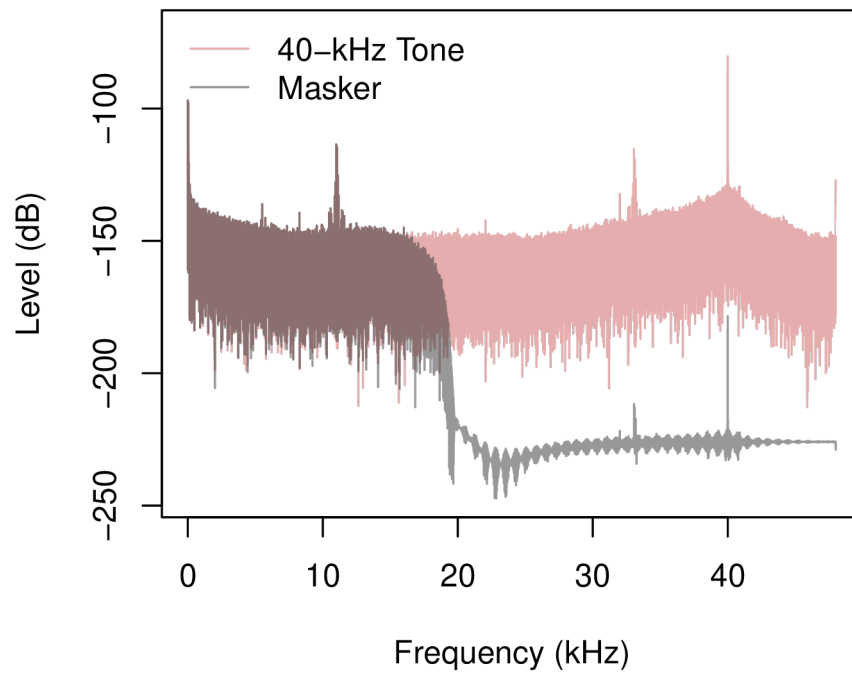


Figure S1: Spectrum of the 40-kHz US tone, and of the masker used in the behavioural US detection task. It should be noted that the two large peaks visible in the spectrum of the US recording around 11, and 33 kHz, as well as the smaller peaks around 5.5, 8.3, and 22 kHz were also present in recordings taken in the soundproof booth while the US speakers were not playing, so they are unrelated to the presentation of the US tone. Some of these peaks are also present in the masker, and may have been audible. However, given that the masker was presented during both the interval containing the US tone, and the interval without the US tone, their presence could not give a cue to the presence/absence of the US tone.

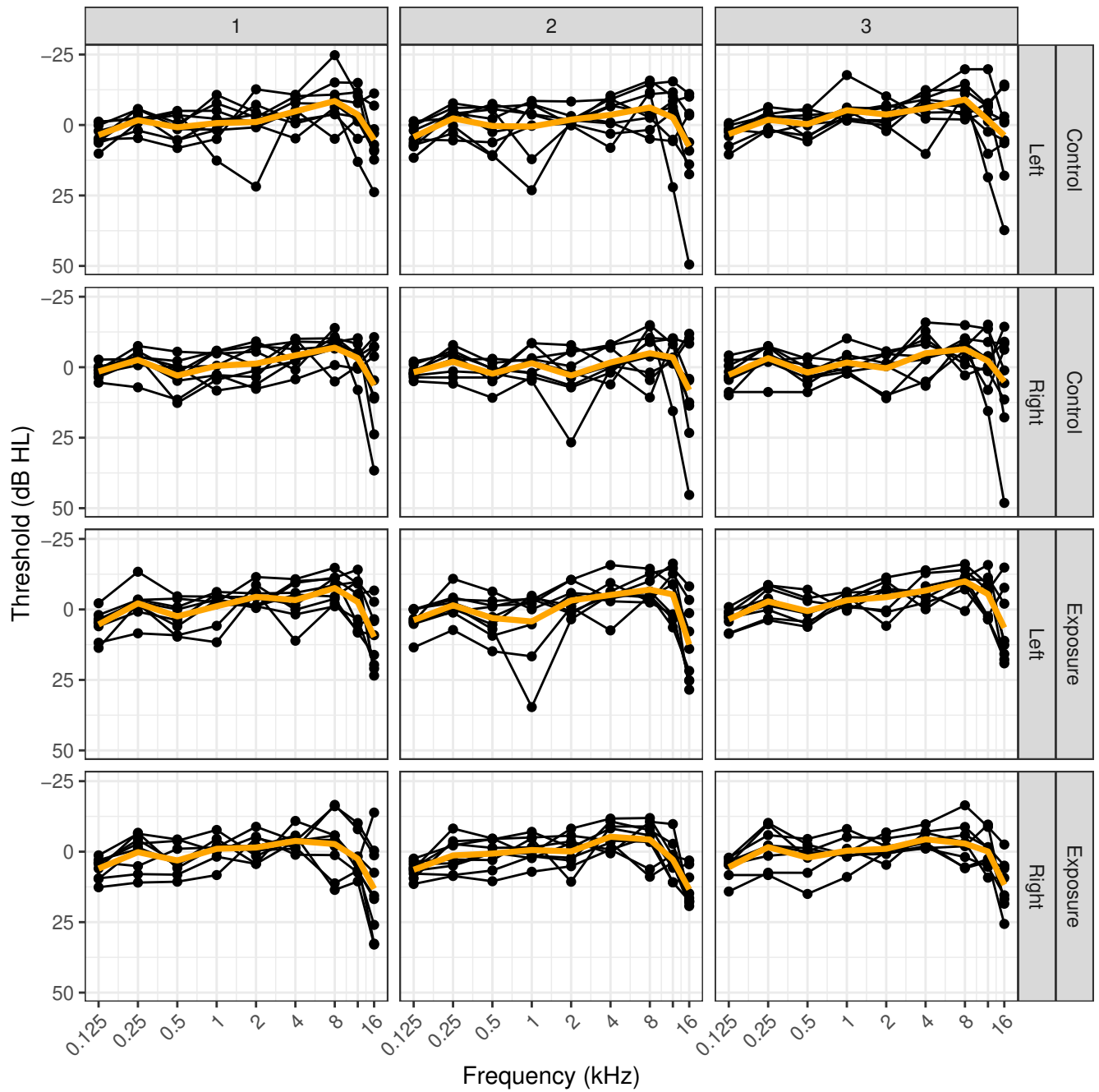


Figure S2: Audiograms for each participant as a function of session number (1, 2, or 3), ear (left, or right), and group (exposure, or control). The orange line shows the average for each panel.

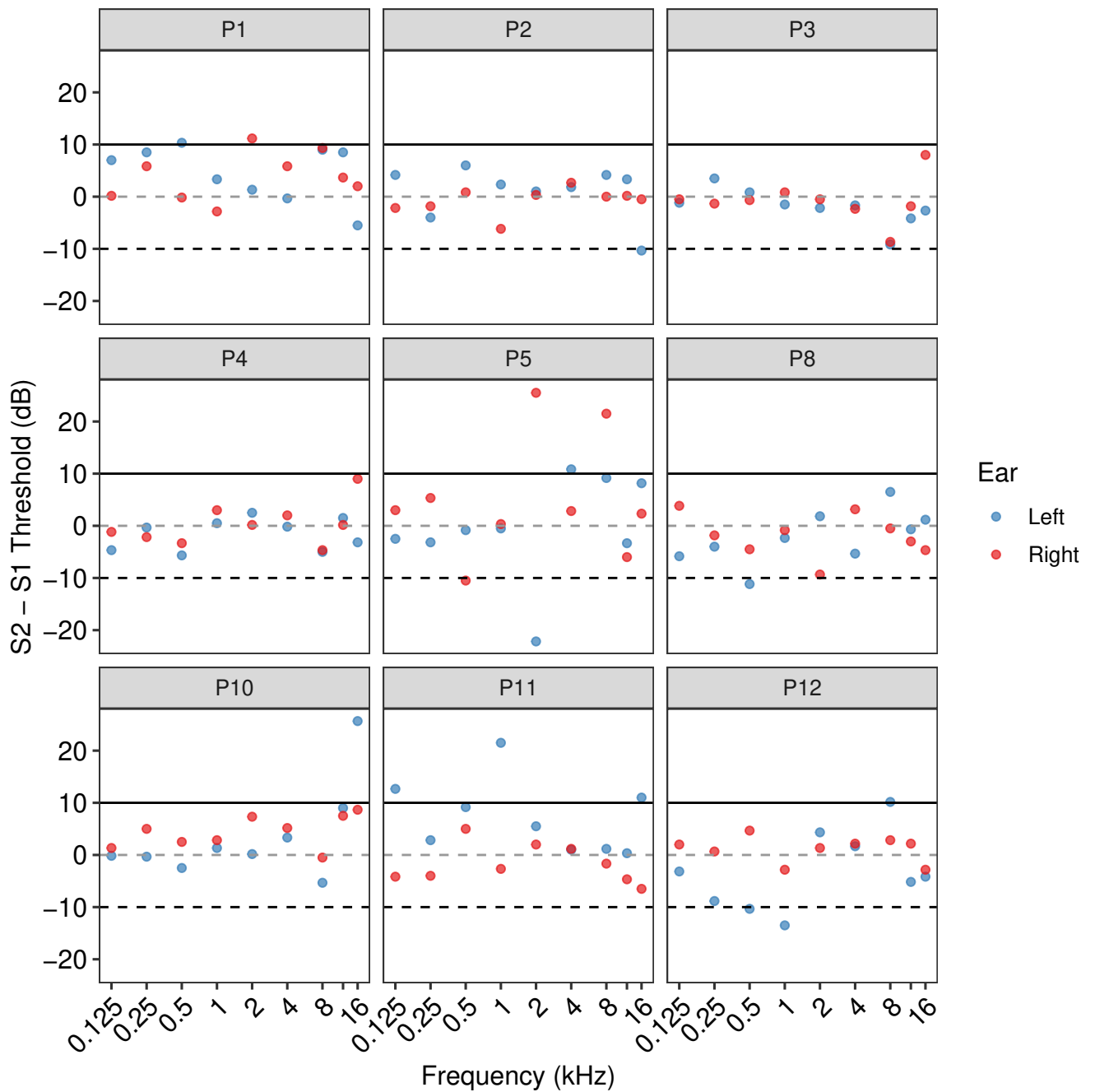


Figure S3: Differences in audiometric thresholds between S2 and S1 for each participant of the control group. Points above the solid line indicate estimated losses of sensitivity > 10 dB. Points below the dashed line indicate estimated gains of sensitivity > 10 dB.

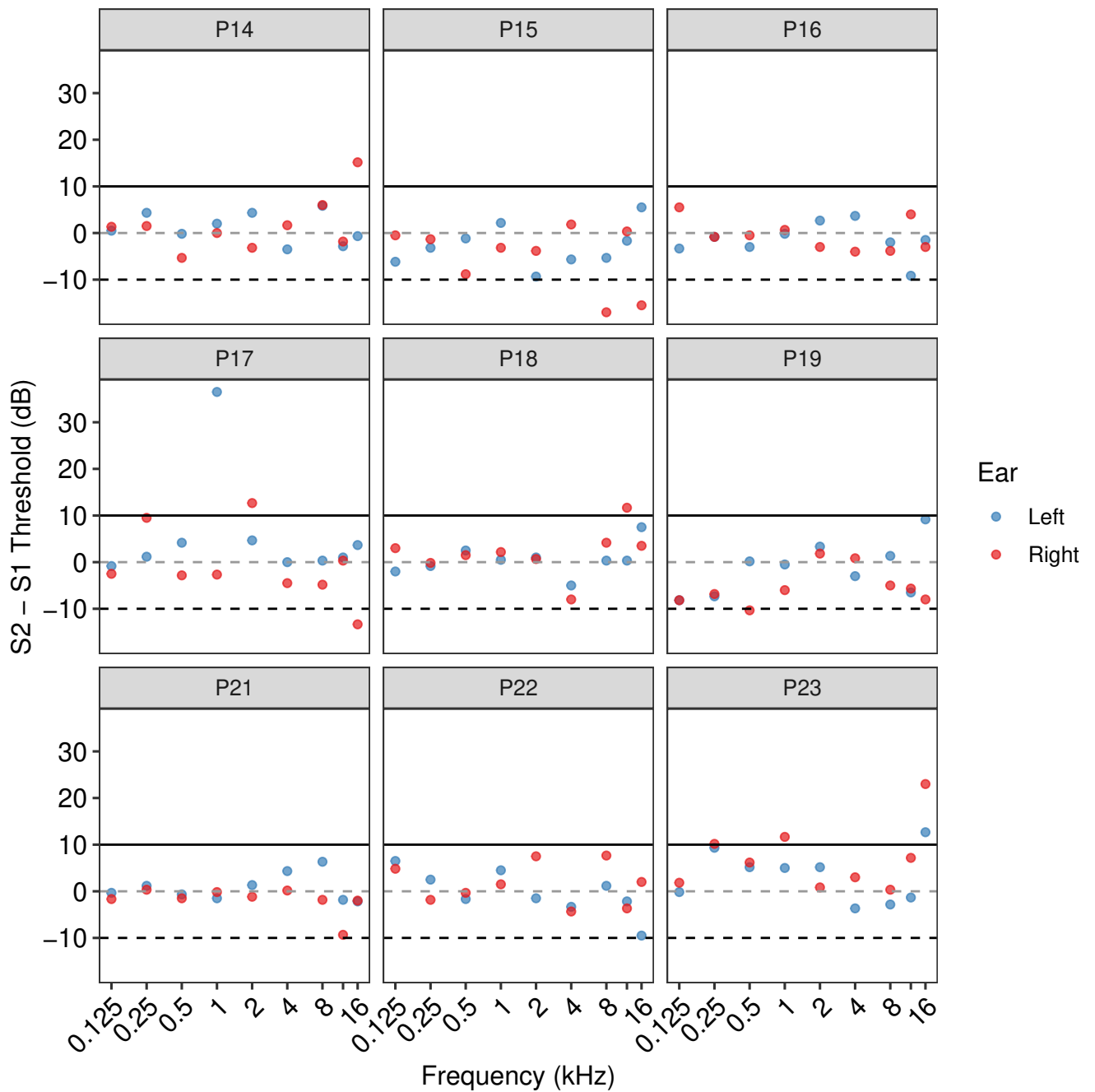


Figure S4: Differences in audiometric thresholds between S2 and S1 for each participant of the exposure group. Points above the solid line indicate estimated losses of sensitivity > 10 dB. Points below the dashed line indicate estimated gains of sensitivity > 10 dB.

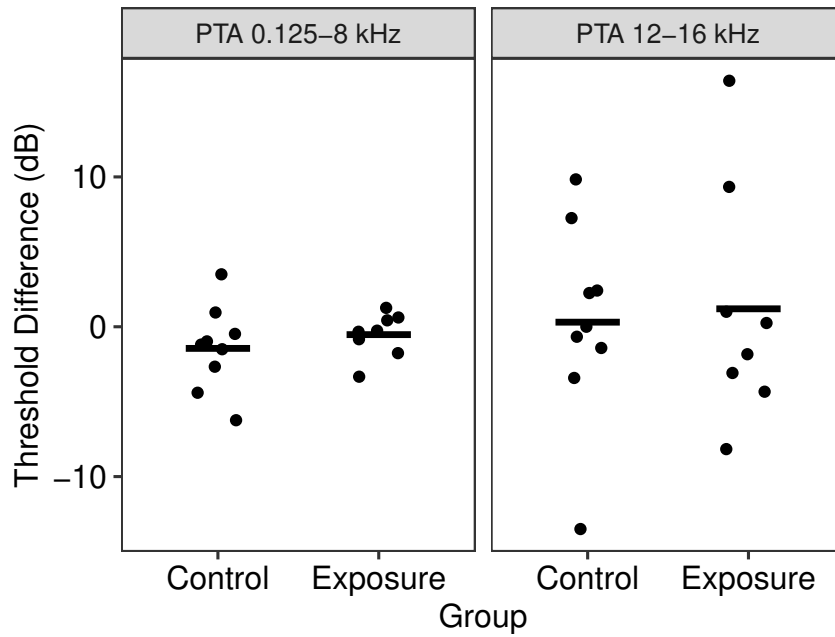


Figure S5: Difference in audiometric thresholds between the left and right ear, between session 3 and session 1: $(T_{L3} - T_{L1}) - (T_{R3} - T_{R1})$, where T refers to the threshold, the first subscript indicates the ear, and the second subscript the session number. An *increase* in the threshold difference in the exposure group would indicate a relative post-exposure loss of sensitivity in the left (exposed) ear compared to the right ear. Points plot individual listeners' data. Segments plot group averages.

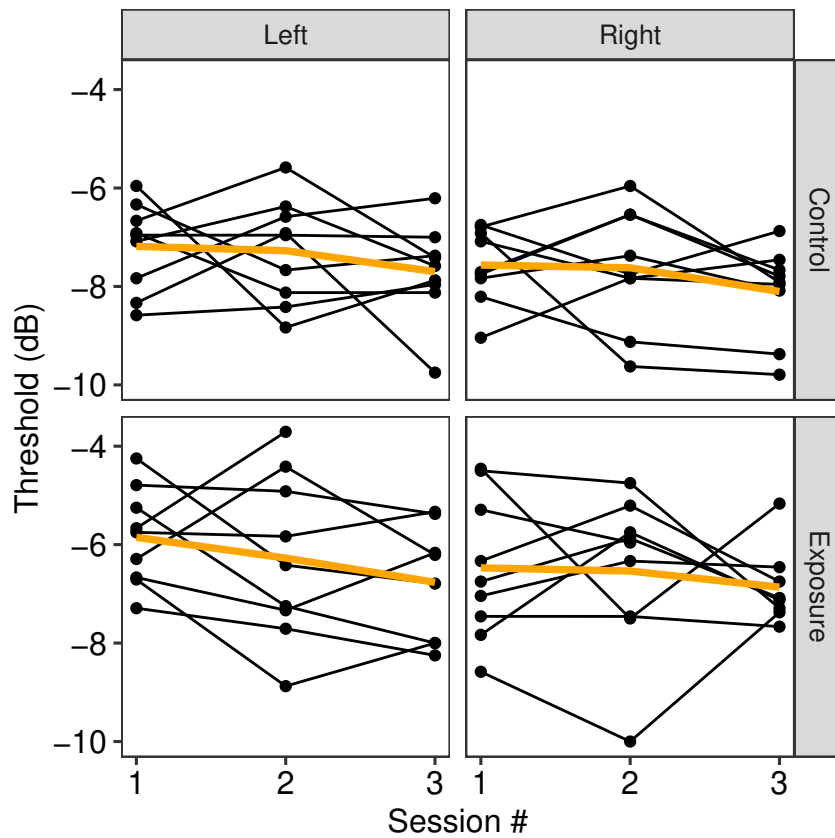


Figure S6: Threshold in the DTT task for each participant, as a function of session number. Results for each group and ear are shown in different panels. The orange line shows the average for each panel.

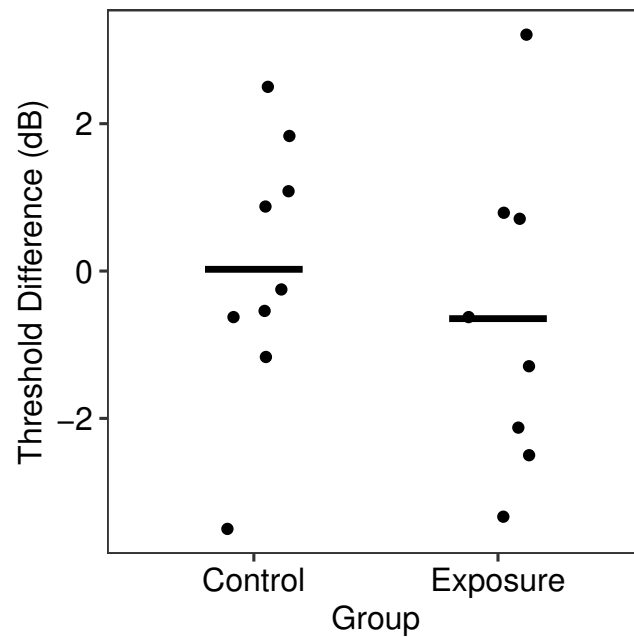


Figure S7: Difference in DTT thresholds between the left and right ear, between session 3 and session 1: $(T_{L3} - T_{L1}) - (T_{R3} - T_{R1})$, where T refers to the threshold, the first subscript indicates the ear, and the second subscript the session number. An *increase* in the threshold difference in the exposure group would indicate a relative post-exposure performance drop for the left (exposed) ear compared to the right ear.

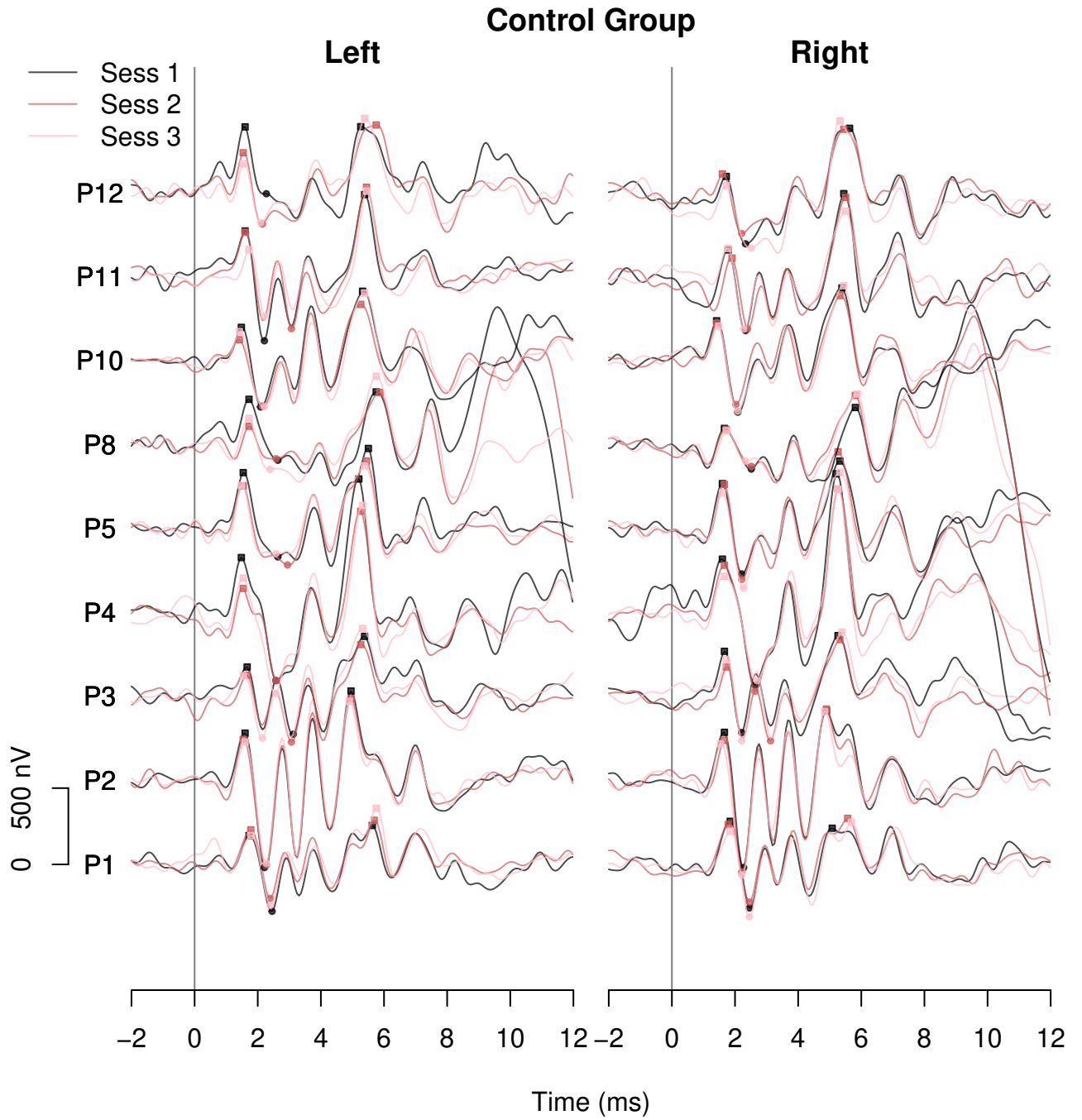


Figure S8: ABR waveforms for participants of the control group.

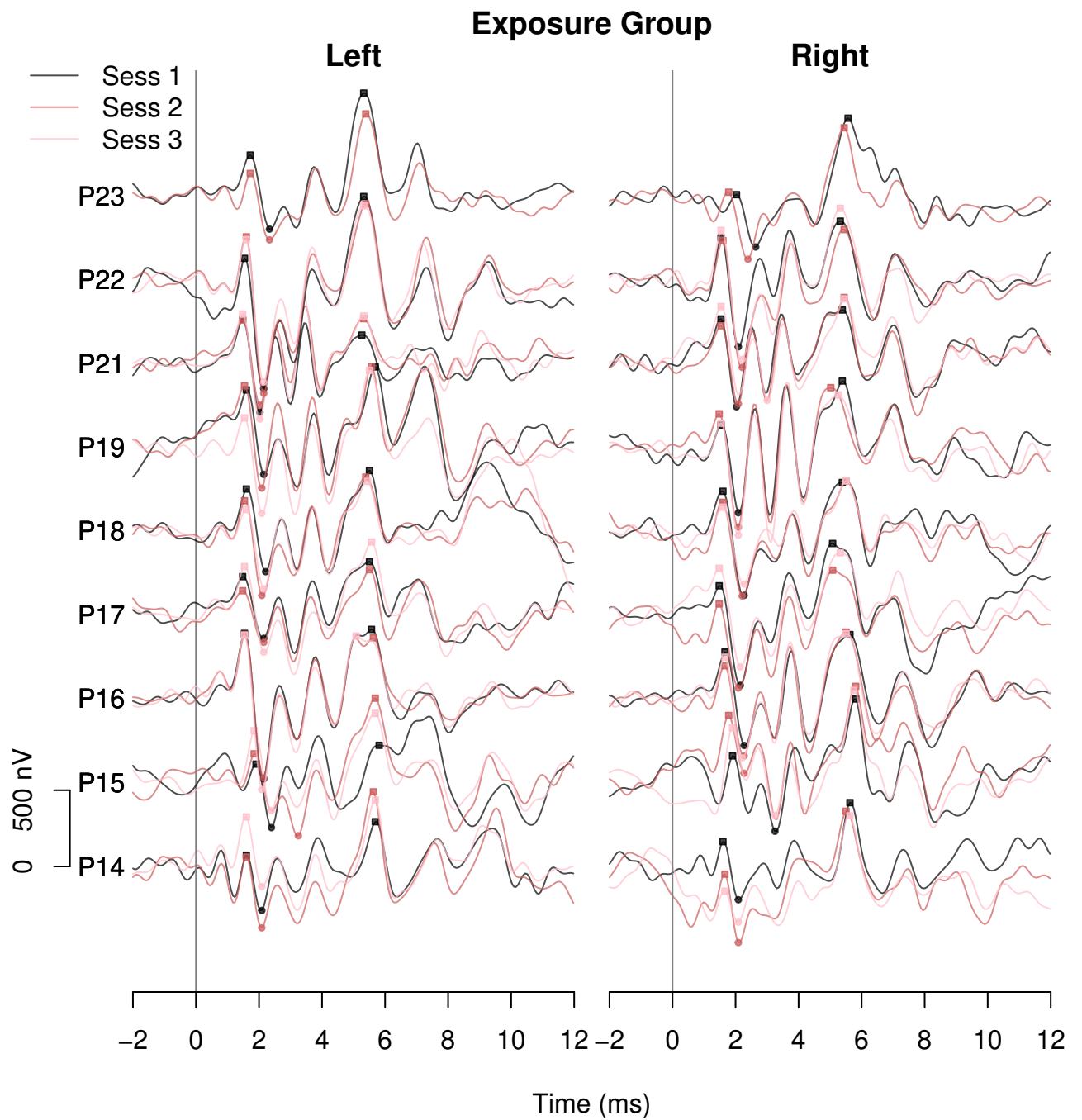


Figure S9: ABR waveforms for participants of the exposure group.

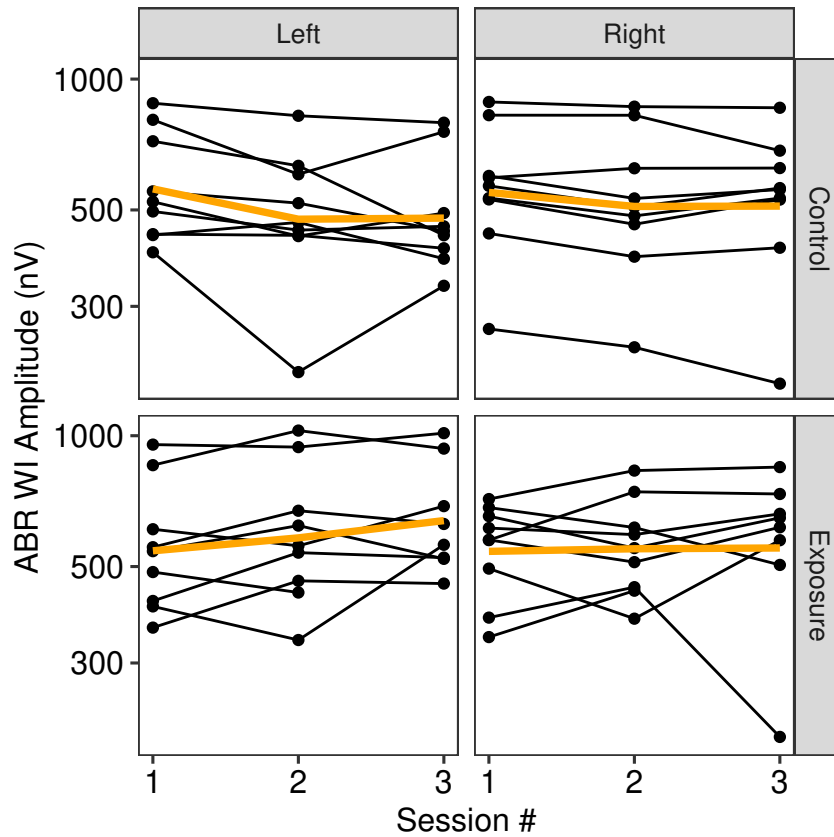


Figure S10: Wave I ABR amplitudes for each participant, as a function of session number. Results for each group and ear are shown in different panels. The orange line shows the geometric average for each panel.

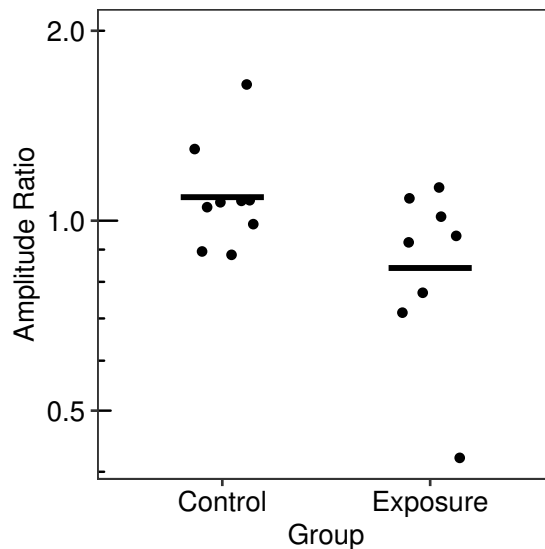


Figure S11: ABR wave I amplitude ratio between the right and left ear, between session 3 and session 1: $(A_{R3}/A_{R1})/(A_{L3}/A_{L1})$, where A refers to the amplitude, the first subscript indicates the ear, and the second subscript the session number. An *increase* in the amplitude ratio in the exposure group would indicate a relative post-exposure wave I amplitude decrease in the left (exposed) ear compared to the right ear.

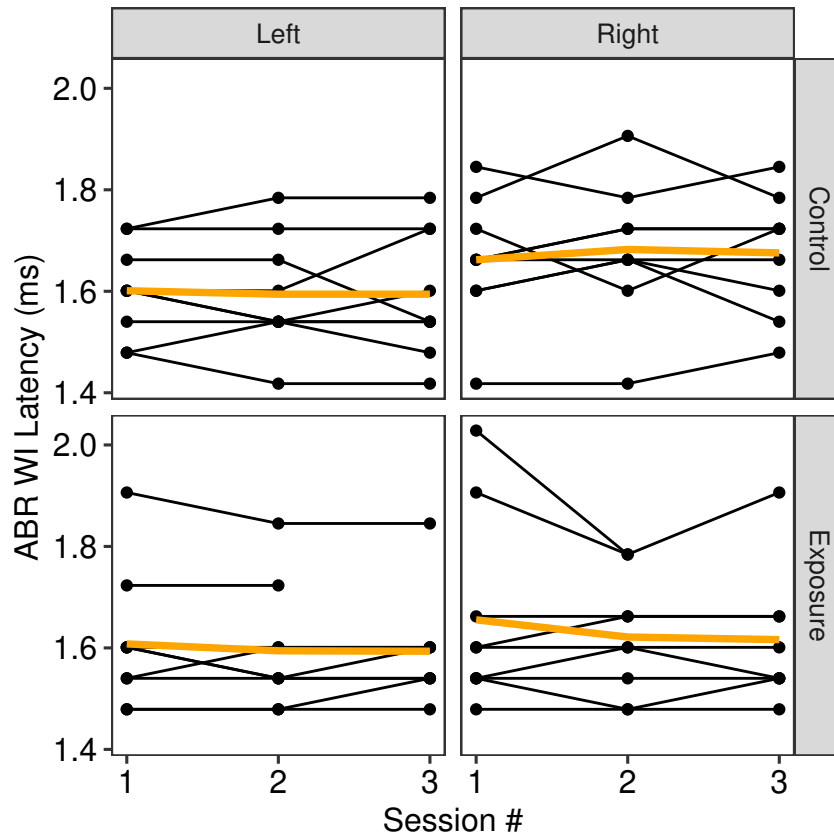


Figure S12: Wave I ABR latencies for each participant, as a function of session number. Results for each group and ear are shown in different panels. The orange line shows the average for each panel.

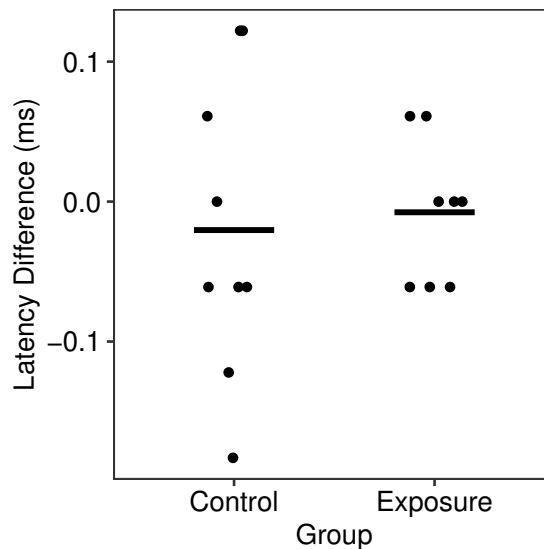


Figure S13: ABR wave I latency difference between the left and right ear, between session 3 and session 1: $(T_{L3} - T_{L1}) - (T_{R3} - T_{R1})$, where T refers to the latency, the first subscript indicates the ear, and the second subscript the session number. An *increase* in the latency difference in the exposure group would indicate a relative post-exposure wave I latency increase in the left (exposed) ear compared to the right ear.

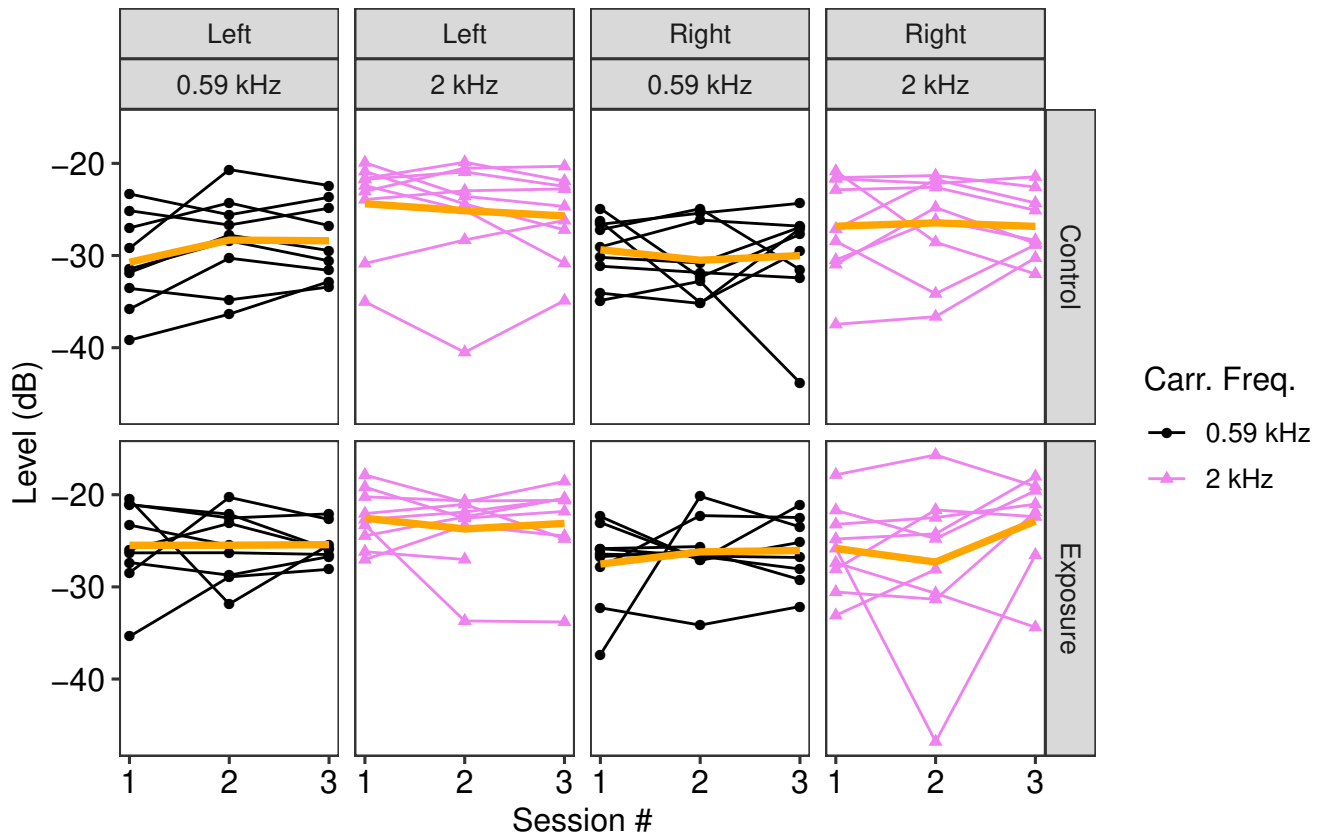


Figure S14: FFR levels for each participant at the modulation frequencies of the carriers, as a function of session number. Results for each group, ear, and carrier frequency are shown in different panels. The orange line shows the average for each panel.

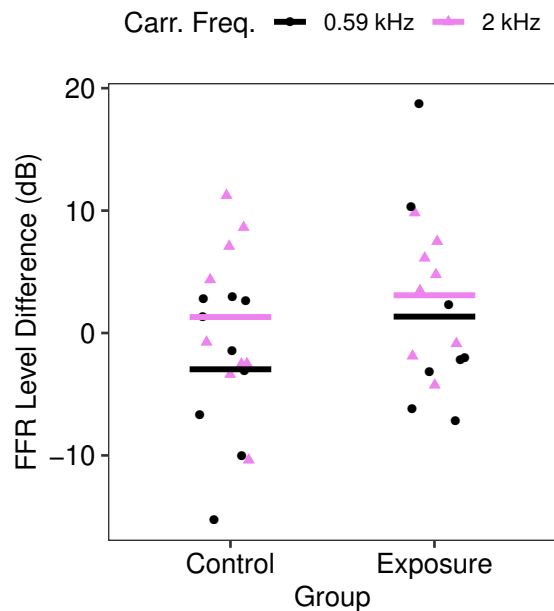


Figure S15: Difference in FFR level between the right and left ear, between session 3 and session 1 ($M_{R3} - M_{R1} - (M_{L3} - M_{L1})$), where M refers to the level, the first subscript indicates the ear, and the second subscript the session number. An *increase* in the level difference in the exposure group would indicate a relative post-exposure decrease in FFR level for the left (exposed) ear compared to the right ear.

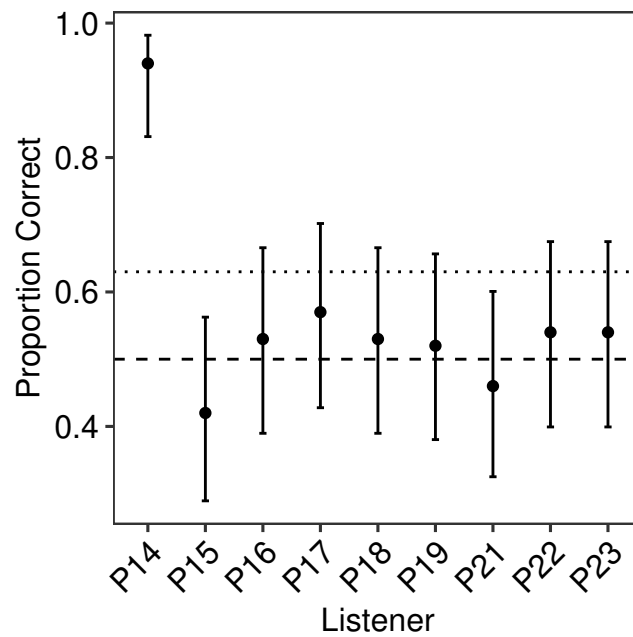


Figure S16: Proportion of correct responses in the detection of the 120 dB SPL ultrasound tone. The dashed line marks chance level. The dotted line marks the threshold for declaring significantly greater than chance level performance after correction for multiple comparisons. The error bars enclose 95% confidence intervals (corrected for multiple comparisons).

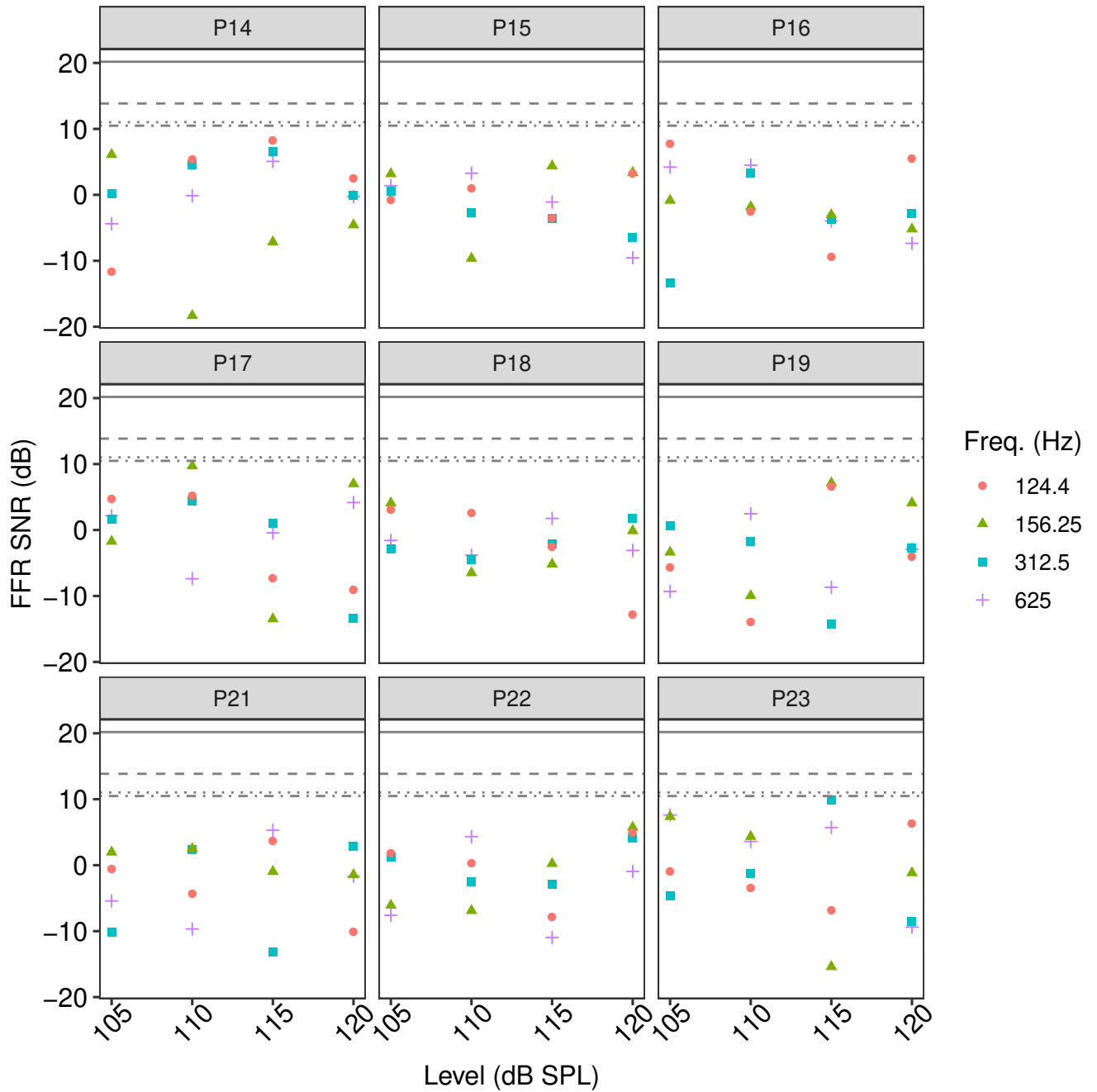


Figure S17: FFR SNR at subharmonic frequencies, and at the modulation frequency of the ultrasound tone for each participant of the exposure group. The lower dash-dotted line marks the SNR threshold for significant signal detection (after accounting for multiple comparisons) for the 124.4 Hz frequency (based on 5-second segments and 5 noise bins on each side). The dotted line marks the SNR threshold for significant signal detection for the 156.25 Hz frequency (based on 4-second segments and 4 noise bins on each side). The dashed line marks the SNR threshold for significant signal detection for the 312.5 Hz frequency (based on 2-second segments and 2 noise bin on each side). The upper solid line marks the SNR threshold for significant signal detection for the 625 Hz frequency (based on 1-second segments and 1 noise bin on each side).

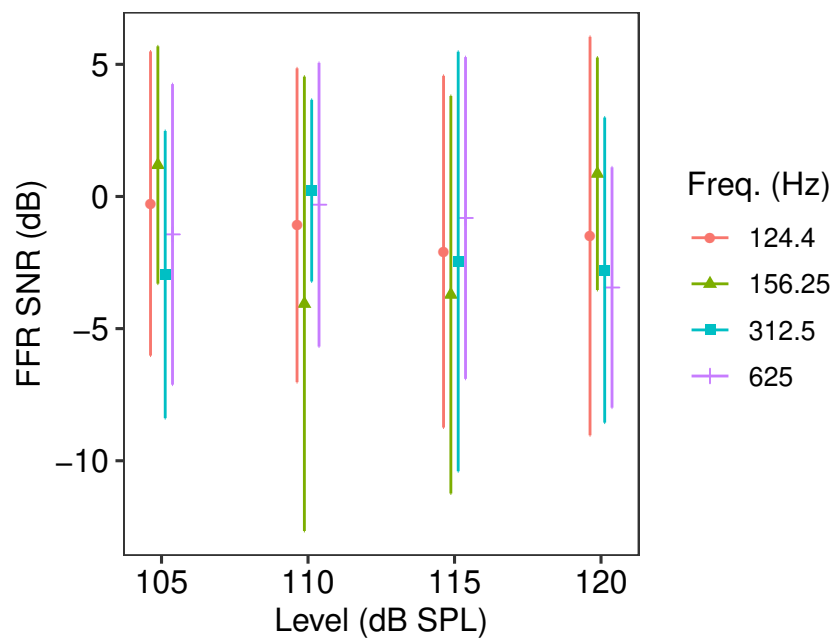


Figure S18: Across participant average FFR SNR at subharmonic and modulation frequencies of the ultrasound tone. The error bars mark ± 1 s.d.

Supplementary tables referenced in the main manuscript

Variable	Corrected 95% CI (one-tailed)	Uncorrected 99% CI (two-tailed)	Bayesian HDI
PTA _{0.125-8}	-2.16-Inf	-2.37-4.2	-3.07-4.83
PTA ₁₂₋₁₆	-9.17-Inf	-9.82-11.6	-12.47-14.33
DTT	-3.37-Inf	-3.55-2.21	-4.26-2.92
ABR WI Log-Amp.	-0.64-Inf	-0.66-0.15	-0.76-0.24
ABR WI Lat.	-0.1-Inf	-0.11-0.13	-0.13-0.16
FFR _{0.59}	-6.53-Inf	-7.25-15.85	-9.96-18.49
FFR ₂	-6.31-Inf	-6.83-10.38	-8.43-12.64

Table S1: Interval estimates for the changes between S1 and S3 for the dependent measures analyzed in the study. The first column shows 95% one-sided CIs corrected for multiple comparisons. The second column show uncorrected 99% CIs. The third column shows 99% Bayesian HDIs.

Changes from the pre-registered protocol

- Although the pre-registered protocol specified testing 10 participants per group, due to time constraints it was not possible to continue data collection to achieve this goal.
- The protocol specified a delay of one to three days between S1 and S-US, and between S-US and S2, and a delay of one week between session S2 and S3. Because participants were occasionally unable to attend a scheduled session it was not possible to follow exactly the planned schedule for each participant.
- Exclusion criteria in the pre-registered protocol included audiometric thresholds > 20 dB HL at any octave frequency between 0.125 kHz and 8 kHz (inclusive) in either ear. One participant of the control group had an estimated threshold of 21.8 dB HL for the left ear at 2 kHz in her first session. However, the standard deviation of the turnpoints of the adaptive track used to estimate this threshold was high (5.14 dB). Because of this, and because the rest of the audiogram appeared normal we assumed that this high threshold was likely due to attentional lapses in the block of trials used to estimate it, and the participant was allowed to proceed onto the other sessions. Her threshold estimates for the left ear at 2 kHz in the remaining sessions were normal, confirming our suspicion that the high threshold estimated in the first session was indeed due to attentional lapses, and her data have been included in the analyses.
- The fact that the US speakers made an audible noise below the US frequency range was only discovered after submission of the pre-registration protocol, therefore the use of the masking noise in the behavioural US detection task is not mentioned there.
- The protocol for the behavioural US detection task specified that the US tone would be amplitude modulated at a frequency of 124.4 Hz. However, recordings of this amplitude modulated tone showed a component at the modulation frequency, possibly generated by modulation distortion in the air. This component was clearly audible. For this reason it was decided to use an unmodulated US tone instead.
- The pre-registration plan for the ultrasound EEG test specified performing an FFT on each 10-min block. However, to achieve a better signal-to-noise ratio shorter segments of the recording were averaged. The results obtained with this analysis were nonetheless qualitatively similar to the ones obtained with the pre-planned analysis, and did not change the study conclusions.

Supplementary methods

ABR wave I peak-peaking algorithm

The latency of the wave I peak was first identified in the grand-average waveform (obtained by averaging across participants from both groups) within a time window centred at a latency of 1.6 ms, and bounds set at ± 0.51 ms. These bounds correspond to ± 3 standard deviations of the ABR wave I latency reported by Issa and Ross [1]. The grand-average wave I peak was identified by selecting the highest local maximum in the search window. The wave I peaks were then searched in the individual subject waveforms within a search window centred at the grand-average wave I peak latency, and with bounds of ± 0.51 ms of the grand-average peak latency. Peaks were identified by selecting the highest local maximum in the search window, or the highest absolute point if no local maxima were present in the search window. Wave I amplitudes were measured from peak to trough. Troughs were identified by selecting the lowest local minimum in a search window going from 0.25 to 1.5 ms from the estimated peak latency, or the lowest absolute point if no local minima were present in the search window.

Supplementary results

Test-retest repeatability

Audiometry

Figure S19 shows the average absolute threshold difference between S1 and S2, and between S1 and S3, across participants from both groups, for each test frequency. Average absolute threshold differences were generally less than 5 dB, although they were higher at 16 kHz, where they reached 7 dB. The absolute threshold differences in this study were higher than those reported in a recent study by John *et al.* [2]. The higher absolute threshold differences found in this study may be partly due to the fact that test and retest were performed on different days, while in the John *et al.* study they were performed within the same day. Another difference between the two studies is that John *et al.* used a modified Hughson-Westlake clinical procedure to estimate thresholds, while in the current study a forced-choice procedure with a transformed up-down adaptive track was used.

Marshall *et al.* [3] measured detection thresholds for a pure tone in quiet using a forced-choice adaptive task, and a clinical procedure on nine listeners for ten blocks. They found that test-retest reliability, assessed by calculating the standard deviation across threshold estimates for each participant (intra-subject SD) was lower for the forced-choice adaptive task than for the clinical procedure. The average intra-subject SD for the forced-choice adaptive task in quiet was 2.2 dB. The average intra-subject SD for each condition of our study is shown in Figure S20. At the same test frequency used by Marshall *et al.* [3] the intra-subject SD was 2.3 dB for the right ear, but it was considerably higher (4.8 dB) for the left ear. It was also higher at most other frequencies in the clinical frequency range. The most likely reason for the higher intra-subject SDs observed in our study is that some listeners occasionally had high lapse rates. Listener's motivation is a factor known to affect psychophysical performance [4], but difficult to control, and we suspect that the occasionally high lapse rates may be due to this.

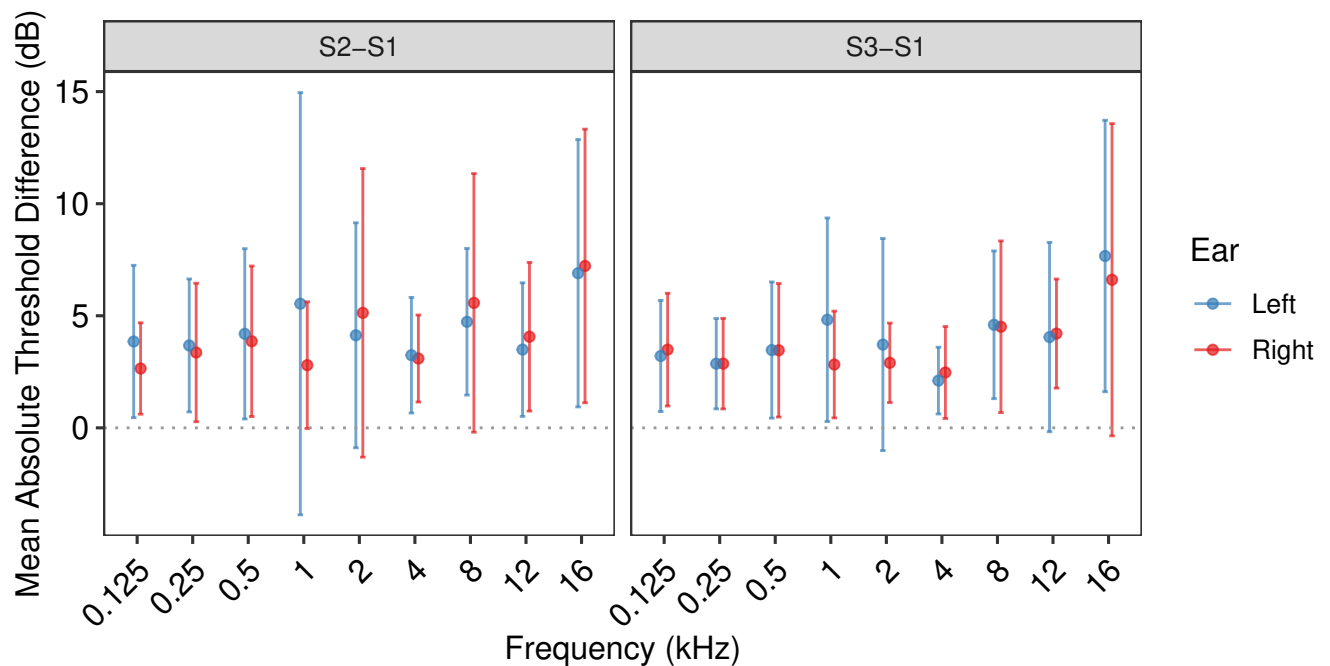


Figure S19: Average absolute test-retest differences in audiometric thresholds between session 1 and session 2 (left panel), and session 1 and session 3 (right panel). Averages were computed across participants from both the control and exposure groups. The error bars represent ± 1 s.d.

We ran some Monte Carlo simulations of a virtual listener performing the forced-choice procedure with the adaptive track parameters (step size, number of turnpoints, etc...) used in the current study to investigate how reliability would be affected by varying the lapse rate. The virtual listener had a logistic psychometric function, with a 70.7% correct point of 0 dB HL, and a slope of 3.7 dB, which was typical of the slopes found by fitting psychometric functions to the data of this study. The results of the simulations showed that for a virtual listener with a 0% lapse rate the absolute threshold differences calculated on 1,000 random samples drawn with resampling from 1,000 Monte Carlo simulations was 1.1 dB, with an SD of 1.1. The absolute threshold difference

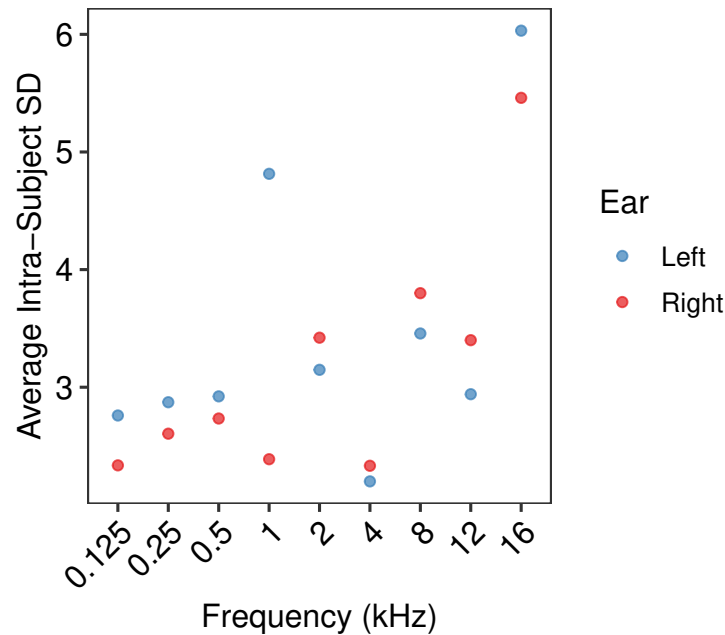


Figure S20: Average intra-subject standard deviations for audiometric threshold estimates measured in the three sessions. Averages were computed across participants from both the control and exposure groups.

71 was still below 1.5 dB for a virtual listener with 1 or 2% lapse rates, but increased to 4 dB for a virtual listener
 72 with a 5% lapse rate. Likewise the SD of the threshold estimates was only 1.3 dB for a virtual listener with a
 73 0% lapse rate, increased to 1.49 dB for a virtual listener with a 2% lapse rate, and then reached 4.4 dB for a
 74 virtual listener with a 5% lapse rate.

75 It should be noted that while a few listeners showed apparent (positive or negative) large threshold shifts,
 76 > 10 dB, at multiple test frequencies, the majority of the listeners either did not show large threshold shifts,
 77 or showed them only in 1 or 2 of the 18 different conditions (see Figures S3, and S4). Furthermore, when the
 78 data were averaged across the two frequency ranges of interest (clinical, and extended high frequency range),
 79 the absolute mean threshold differences were much lower. These can be seen in Table S2, which lists absolute
 80 threshold differences for all the dependent measures analysed in the study. Likewise the mean intra-subject SDs
 81 were much lower when the data were averaged across the two frequency ranges of interest. These can be seen
 82 in Table S3 which lists intra-subject SDs for all the dependent measures analysed in the study.

83 Another way to measure repeatability is the intraclass correlation coefficient (ICC). The ICC measures within-
 84 subject variability across sessions relative to between-subject variability, and it can be low if interindividual
 85 differences are small in the population or sample observed. ICCs calculated with the rptR package [5] for all the
 86 dependent measures analysed in the study are given in Table S4. The ICC for the average PTA in the extended
 87 high frequency range was higher than the ICC for the average PTA in the clinical frequency range, despite the
 88 fact that intrasubject SDs were lower in the clinical than in the extended high frequency range. The reason for
 89 this is that between-subject variability was higher in the extended than in the clinical frequency range.

Variable	S1-S2 Left	S1-S2 Right	S1-S2 Mean	S1-S3 Left	S1-S3 Right	S1-S3 Mean
PTA _{0.125–8}	2.63	2.3	2.46	2.15	1.25	1.7
PTA _{12–16}	3.87	4.87	4.37	4.46	4.5	4.48
DTT	1.19	1.13	1.16	1.07	0.88	0.98
ABR WI Log-Amp.	0.16	0.13	0.14	0.15	0.14	0.15
ABR WI Lat.	0.03	0.06	0.05	0.05	0.03	0.04
FFR _{0.59}	3.71	3.48	3.59	2.73	3.71	3.22
FFR ₂	2.75	4.12	3.43	3.24	3.76	3.5

Table S2: Mean absolute differences between S1 and S2, and between S1 and S3 for the dependent measures analysed in the study. Values are given for the left ear, the right ear, and the mean of the left and right ear values.

Variable	Left	Right	Mean
PTA _{0.125–8}	2.03	1.56	1.79
PTA _{12–16}	3.5	3.69	3.6
DTT	0.83	0.82	0.82
ABR WI Log-Amp.	0.12	0.11	0.12
ABR WI Lat.	0.03	0.04	0.04
FFR _{0.59}	2.23	2.72	2.48
FFR ₂	2.04	3.34	2.69

Table S3: Mean intra-subject SDs for the dependent measures analysed in the study. Values are given for the left ear, the right ear, and the mean of the left and right ear values.

Variable	ICC	95% CI
PTA _{0.125–8}	0.535	0.272–0.695
PTA _{12–16}	0.717	0.502–0.838
DTT	0.487	0.234–0.661
ABR WI Log-Amp.	0.742	0.526–0.849
ABR WI Lat.	0.813	0.638–0.896
FFR _{0.59}	0.536	0.291–0.701
FFR ₂	0.554	0.308–0.719

Table S4: ICCs for the dependent measures analysed in the study. The second column shows the 95% ICC confidence intervals.

DTT

Both mean absolute across-session differences (Table S2), and mean intra-subject SDs (Table S3) were relatively small, indicating good reliability of the measure. The ICC (Table S4), however, was modest due to the fact that between-subject variability was low.

ABR

Both wave I ABR amplitudes and latencies were remarkably stable across sessions, as indexed by the mean absolute differences (Table S2), mean intra-subject SDs (Table S3), and ICCs (Table S4). The log-amplitude mean absolute differences are easier to interpret when converted to ratios by exponentiating. When converted to ratios they ranged from 1.14 to 1.17. Likewise average intra-subject SDs are easier to interpret when converted to ratios (or equivalently when calculated as geometric averages of the geometric intra-subject SDs). These ranged from 1.12 to 1.13. The ICCs were similar in size to those obtained in two recent study of supra-threshold ABR test-retest reliability [6, 7].

FFR

Reliability of the FFR measures was only moderate, as indexed by the mean absolute differences (Table S2), mean intra-subject SDs (Table S3), and ICCs (Table S4). A recent study by Guest *et al.* [7] reported high reliability for FFR level in response to AM tones. However, there were several differences in the stimuli and procedures used in this previous study, and the current study, which may explain the lower test-retest reliability observed in the current study. Unlike the previous study we presented the stimuli monaurally, we presented two stimuli simultaneously, and we used AM tones rather than transposed tones. Monaural stimulation leads to lower FFR amplitudes even when the monaural stimuli are presented at higher SPLs to compensate for level differences [8]. At stimulus levels of 75 dB, FFR amplitudes to multiple simultaneous stimuli have been found to be reduced in amplitude compared to when the stimuli are presented individually [9]; these amplitude reductions were largest for the stimulus with the lower carrier frequency. Transposed tones enhance phase locking to the envelope of modulated high-frequency carriers compared to AM tones [10, 11]. Overall these three factors are likely to explain at least in part the lower FFR amplitudes observed in the current study, which resulted in FFR levels being closer to the noise baseline, and likely reduced test-retest reliability. Other differences between the studies may also have played a role in the reduced test-retest reliability observed in the current study. For example, due to space limitations, in the current study it was not possible to recline the chair during the recordings, which may have led to increased myogenic artifacts.

119 **Bayesian model**

120 The JAGS code for the Bayesian model is provided below:

```

121 model {
122   # likelihood
123   for (i in 1:Ntotal) {
124     y[i] ~ dnorm(mu[x[i]], 1/sigma[x[i]]^2)
125   }
126
127   #priors
128   for (j in 1:2) {
129     mu[j] ~ dnorm(meanY , 1/(100*sdY)^2)
130     sigma[j] ~ dunif(sdY/1000 , sdY*1000)
131   }
132   muDiff = mu[1]-mu[2]

```

133 **y** is a vector with the dependent variable. **x** is a vector indicating the group (experimental or control). **meanY**,
 134 and **sdY** are respectively the mean, and the standard deviation of the dependent variable across groups; these
 135 values are used to set vague priors on the scale of the data.

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