Hearing assessment during deep brain stimulation of the central nucleus of the inferior colliculus and dentate cerebellar nucleus in rat

4 Smit JV[a,b,*], Jahanshahi A[b,c], Janssen MLF[b,d], Stokroos RJ[a,b], Temel Y[b,c]

6 [a] Department of Ear Nose and Throat/Head and Neck Surgery, [b] Department of Neuroscience,
7 School for Mental Health and Neuroscience, [c] Department of Neurosurgery, [d] Department of
8 Neurology, Maastricht University Medical Center, The Netherlands

11 *** Correspondence**

12 Jasper Smit

13 Department of Ear Nose and Throat/Head and Neck Surgery

14 Maastricht University Medical Center

15 P. Debyelaan 25, 6202 AZ Maastricht, The Netherlands

16 Tel. 0031-43-3881033, Fax 0031-43-3875580

17 E-mail: jasper.smit@maastrichtuniversity.nl

Abstract

Background. Recently it has been shown in animal studies that deep brain stimulation (DBS) of auditory structures was able to reduce tinnitus-like behavior. However, the question arises whether hearing might be impaired when interfering in auditory-related network loops with DBS.

Methods. The auditory brainstem response (ABR) was measured in rats during high frequency stimulation (HFS) and low frequency stimulation (LFS) in the central nucleus of the inferior colliculus (CIC, n=5) or dentate cerebellar nucleus (DCBN, n=5). Besides hearing thresholds using ABR, relative measures of latency and amplitude can be extracted from the ABR. In this study ABR thresholds, interpeak latencies (I-III, III-V, I-V) and V/I amplitude ratio were measured during off-stimulation state and during LFS and HFS.

Results. In both the CIC and the CNBN groups, no significant differences were observed for all outcome measures.

Discussion. DBS in both the CIC and the CNBN did not have adverse effects on hearing measurements. These findings suggest that DBS does not hamper physiological processing in the auditory circuitry.

Introduction

Deep brain stimulation (DBS) in auditory structures has been performed in animal studies as a treatment for tinnitus (Luo et al., 2012; Smit et al., 2016). The rationale behind this treatment is to interfere with the pathological neuronal activity in the central nervous system and interrupt the network loop that is essential for the persistence of tinnitus (Smit et al., 2015).

The fundamental knowledge of the effect of deep DBS in auditory structures on hearing is essential before applying this treatment in a clinical setting (Smit et al., 2015). It has been shown in rats, using the sound-induced pre-pulse inhibition test with click stimuli, that during high frequency stimulation (HFS) of the external nucleus of the informed colliculus (IC) hearing thresholds did not change (Smit et al., 2016). As far as we know, a more detailed hearing assessment during DBS in auditory structures has not been assessed thus far.

To assess hearing thresholds in more detail, the auditory brainstem response (ABR) was measured in this study. The ABR assesses changes in neural integrity and is commonly used in laboratory animal studies to estimate hearing (Rosahl et al., 2000; Turner et al., 2006). In humans, ABRs are used in daily practice to assess possible hearing loss of a retrocochlear origin (Stockard and Rossiter, 1977).

Two structures were targeted in this study, the central nucleus of the IC (CIC) and the dentate cerebellar nucleus (DCBN). The CIC is the principal auditory part of the IC and has a well-defined tonotopy (Aitkin and Moore, 1975; De Martino et al., 2013). In animal models of tinnitus, the IC shows tonotopic reorganization, increased spontaneous firing rate, increased bursting activity and increased neural synchrony (Bauer et al., 2008; Chen and Jastreboff, 1995; Robertson et al., 2013; Wang, Ding and Salvi, 2002). A recent study showed that HFS of the

63   external nucleus of the inferior colliculus (IC) in rats decreased tinnitus-like behavior (Smit et

64   al., 2016). The cerebellum is a structure that is not involved in the auditory pathways but is

65   associated with tinnitus (Brozoski, Ciobanu and Bauer, 2007; Osaki et al., 2005; Sedley et al.,

66   2012; Shulman and Strashun, 1999). It was demonstrated that ablation of the paraflocculus

67   completely diminished tinnitus in rats (Bauer et al., 2012). The majority of fibers in the

68   cerebellum, including the paraflocculus, originate from the deep cerebellar nuclei, especially the

69   DCBN, which is the largest (Gayer and Faull, 1988; Gould, 1979). Therefore, the CIC and the

70   DCBN could be considered as respectively an auditory and a non-auditory potential DBS target

71   for the treatment of tinnitus.

72   DBS can be performed with low frequency stimulation (LFS), which mainly has an excitatory

73   effect, and as HFS, which generally is described as a global inhibitory effect similar as ablation

74   (Benabid et al., 1998; Breit, Schulz and Benabid, 2004; Dostrovsky and Lozano, 2002).

75   Following ablation of IC in animals models, decreased amplitude and latency of peak V have

76   been found (Achor and Starr, 1980; Buchwald and Huang, 1975; Durrant et al., 1994; Kaga,

77   Shinoda and Suzuki, 1997). Peak V is the last of the five peaks of the ABR and represents neural

78   activity of the IC. Because of a high variability in amplitude among subjects, the V/I amplitude

79   ratio is a more consistent measure than the absolute value (Musiek et al., 1984; Musiek, Reeves

80   and Baran, 1985). The relative measures of the latencies are the interpeak latencies (I-III, III-V,

81   I-V) which represent the central transmission latency best (Eggermont and Don, 1986; Picton et

82   al., 1977; Squires, Chu and Starr, 1978). There is little evidence that stimulation of cerebellar

83   structures has influence on the ABR (Crispino and Bullock, 1984).

84   We hypothesize that for CIC stimulation, the V/I amplitude ratio of the ABR would be lower and

85   the I-V or III-V interpeak latencies would be prolonged during HFS and not during LFS of the

86   CIC. Our hypothesis is that stimulating a non-auditory structure such as the DCBN does not have

87   any influence on the ABR.

89 **Methods**

90 *Animals*

91 Male rats (Sprague Dawley, 250-300 g, Charles River, The Netherlands) were housed

92 individually under conditions of constant room temperature and humidity with a reversed

93 12u/12u light/dark cycle and had free access to water and food. The Animal Experiments

94 Committee of the Maastricht University approved the experiments (approval reference number

95 2012-069).

96 *Surgical procedure*

97 Subcutaneous electrodes were implanted for ABR recordings and during the same surgery DBS

98 electrodes were implanted in the brain (Figure 1). Animals were anesthetized by intraperitoneal

99 administration of ketamine (90 mg/kg) and xylazine (10 mg/kg). The head of the rats was

100 immobilized in a stereotactic apparatus (Stoelting Co, Wood Dale, Illinois) with mouth and blunt

101 ear-bars. Permanent Teflon-coated stainless steel electrodes were subcutaneously implanted. One

102 wire electrode was subcutaneously tunneled to the mastoid and a second wire electrode was

103 attached to a screw on the vertex. Based on coordinates from a stereotactic atlas (Paxinos and

104 Watson, 2007), bilateral electrodes (Technomed, Beek, The Netherlands) were inserted in the

105 CIC (bregma -8.8, depth 4.5, interspace 3.8) or in the DCBN (bregma -11.5, depth 6.5, interspace

106 6.8). The postoperative recovery time was one week.

107 *Deep brain stimulation*

108 DBS was performed with bipolar, concentric electrodes using monophasic rectangular pulses.

109 The electrical stimulus pulses were created by an A310 accupulser and an A360 stimulus isolator

110   (World Precision Instruments, Berlin, Germany). During DBS, stimuli were given with a

111   frequency of 100Hz (HFS) and 10Hz (LFS) with an amplitude of 100 μA and a pulse width of 60

112   μs. Electrodes are gold-plated with platinum-iridium inner wire (negative contact) and stainless

113   steel outer part (positive contact). The inner and outer electrodes are insulated except for a 75 μm

114   exposed tip (Tan et al., 2010).

115   Rats were divided in two groups, one group received implantation of electrodes in the CIC (n=5)

116   and the other group in the DCBN (n=5). In the off-stimulation state, designated as the control

117   situation, no electrical stimulation was given. During stimulation-off state, LFS (10 Hz) and HFS

118   (100 Hz), ABRs were recorded in separate sessions.

119   *Auditory brainstem response*

120   ABR measurements were performed in a random manner of the three situations (off-stimulation,

121   LFS, HFS) with a one week interval. Stimulation was turned on approximately 5 minutes before

122   ABR recordings. HFS consisted of a concentric bipolar electrode using monophasic rectangular

123   pulses, with a frequency of 100 Hz, amplitude of 100 μA per electrode and a pulse width of 60

124   μs (A310 Acupulser, World Precision Instruments, Berlin, Germany). Similar settings were used

125   in a study which showed tinnitus reduction during HFS in rats (Smit et al., 2016). LFS consisted

126   of the same parameters with a frequency of 10 Hz.

127   To achieve anesthesia during ABR recordings, intraperitoneal administration of ketamine (90

128   mg/kg) and xylazine (10 mg/kg) was used, which is preferred over isoflurane when assessing

129   hearing thresholds in rats (Ruebhausen, Brozoski and Bauer, 2012).

130   During the ABR procedure, animals were placed into a sound-attenuating chamber. Cables were

131   plugged into the socket of the head of the animal and connected to the recording device

132   (Powerlab 8/35 connected to a Dual Bio Amp amplifier (ADInstruments, Castle Hill, Australia).

133   An electrode connected to the left hind paw served as the ground.

134   Custrom-made auditory stimuli (10, 16, 24 and 32 kHz) were created with Matlab 2011a

135   (Mathworks, MA, USA) and consisted of 5 ms bursts with a cos^2 rise and fall filter and were

136   played at a rate of 20 per second at decreasing intensities from 90 to 0 dB peSPL with steps of 10

137   dB. To prevent synchronous occurrence of stimulation artifacts with the ABRs, one in 10 stimuli

138   had an interval of 55 ms instead of 45 ms. To gain an approximately similar amount of data after

139   filtering of stimulation artefacts, 500 auditory stimuli were given per intensity in the off-

140   stimulation state, 700 during LFS and 1000 during HFS. Sounds were calibrated with a Bruel &

141   Kjaer 2231 decibel meter with a 4191 microphone (range 2-40 kHz), which was placed at the

142   location of the rat's right ear. Sound intensities are reported as the peak equivalent sound

143   pressure level (peSPL).

144   Auditory stimuli were processed with an external soundcard with a sample rate of 192 kHz

145   (Creative E-MU 0204), amplified with Ultrasonic power amplifier (Avisoft Bioacoustics, Berlin)

146   and played with an Ultrasonic Dynamic Speaker Vifa (Avisoft Bioacoustics, Berlin, Germany) to

147   the right ear. To standardize sound presentation between recording sessions it was monitored that

148   in every session the same position of the rat and the same distance between the loudspeaker and

149   the ear was used (2 cm). The contralateral ear was plugged with modeling clay.

150   Auditory stimuli were digitally triggered. The recordings were done in Labchart Pro 7

151   (ADInstruments, Castle Hill, Australia) at a sample frequency of 20 kHz and raw data were

152   imported into Matlab. With a customized script, the signal was amplified 100,000 times and

153   band-pass filtered (300-3000 Hz). Evoked responses were averaged and data which contained

154   DBS artifacts were automatically removed based on a peak-detection analysis. Using a

155   customized Matlab script, peaks were automatically detected if the signal was above a manual

156   depicted maximal baseline value. Before and after the maximal value of the peak of the artefact

157   2.5ms of data were converted in Not-a-Number (NaN). The ABR and DBS stimuli were not

158   phase-locked so per epoch a different part was converted in NaN. All epochs were averaged to

159   calculate the mean ABR signal (Figure 2B).

160   Two independent blinded observers visually identified ABR thresholds and peaks. In case of

161   disagreement, a third observer was sought and the concordant data were accepted. The auditory

162   threshold was defined as the lowest decibel level (peSPL) of the stimulus, which produced a

163   distinctive ABR.

164   For latency analysis, the five positive peaks were determined at 90 dB peSPL and numbered I-V

165   based on the recordings of vertex upward deflections (for an example see Figure 2C). Latencies

166   of peaks were measured from stimulus onset. Interpeak latency was defined as the time between

167   respective peaks.

168   The amplitude was expressed as the peak-to-peak amplitude ratio of peak V subtracted by peak I.

169   *Electrode localization*

170   Animals were deeply anesthetized with pentobarbital (75 mg/kg) and perfused transcardially

171   with Tyrode's buffer (0.1 M) and fixative containing 4% paraformaldehyde, 15% picric acid and

172   0.05% glutaraldehyde in 0.1 M phosphate buffer (pH 7.6). After post-fixation for 12 hours, the

173   brains were cut to coronal sections using a vibrotome. To assess the electrode localization, the

174   sections containing the target area and the electrode trajectory were stained with hematoxylin-

175   eosin (Merck, Darmstadt, Germany). Definition of anatomic structures was based on the

176   stereotactical atlas (Paxinos and Watson, 2007).

177   *Statistical analysis*

178   Dependent data were analyzed using the Wilcoxon signed-rank Test for two groups and a

179   Friedman test for multiple groups. Since multiple comparisons were made when comparing the

180   stimulation-off state with LFS and HFS, modified p-values (alpha = 0.05) are given as corrected

181   by means of the Holm-Bonferonni sequential correction (Holm, 1979). Data are presented as

182   mean ± standard error of the mean (SEM). All data were analyzed with SPSS (Version 20, IBM,

183   Somers, NY, USA).

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Results

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Electrode localization

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188 Histological evaluation showed that all electrodes were implanted correctly in the target

189 structures (Figures 3A and 3B, respectively).

Hearing thresholds

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191 Hearing response thresholds were determined as the minimal intensity stimulus at which an ABR

192 was evident. Thresholds of different stimulus frequencies (10, 16, 24 and 32 kHz) are depicted in

193 Figure 4A for the CIC group and in Figure 4B for the DCBN group. In one rat two thresholds (10

194 Hz LFS and 32 Hz LFS) were not possible to determine. In both groups, no statistically

195 significant differences were found during HFS and LFS compared to off-stimulation.

Latencies and amplitudes

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197 From all ABRs, 5 distinctive peaks could be determined at 90 dB peSPL (Figure 1). In Table 1

198 the mean interpeak latencies (I-III, III-V and I-V) are shown for different burst frequencies (10,

199 16, 24 and 32 kHz). In both the CIC and the DCBN group, no statistically significant differences

200 were found for high and low frequency DBS compared to no stimulation (Table 1).

201 The V/I amplitude ratio was calculated at all burst frequencies. In both groups, there was no

202 statistical significant difference when comparing no stimulation with HFS and LFS. Appendix 1

203 shows the absolute latencies and interpeak latencies.

204 When looking at the latency and amplitude data, a relation between ABR latencies and

205 amplitudes, with frequencies of burst tones was noticed. For further analysis, we grouped the

206 off-stimulation data of the CIC and DCBN group since only baseline measurements were

207 analyzed. The latency, e.g. of peak I, differed between burst frequencies (X^2(3) = 20.12, p

208 <0.01). The raw data (see Appendix 1) show a shorter latency with increasing frequencies of

209 burst tones. The V/I amplitude ratio does not differ amongst frequencies (X^2(3) = 4.92, p = .178).

210 Amplitudes of peak I did not differ between frequencies (X^2(3) = 3.240, p = .355), but the

211 amplitude of peak V was different between frequencies (X^2(3) = 17.160, p < 0.01). Also peak V

212 amplitude decreases with increasing burst frequency.

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221 **Discussion**

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223 We successfully measured ABRs during stimulation-off state, LFS and HFS. Our results showed

224 that LFS as well as HFS in the CIC and DCBN do not influence ABR thresholds, interpeak

225 latencies and amplitude ratios in rats.

226 *ABR thresholds*

227 The finding that ABR thresholds were not influenced by LFS and HFS suggests that hearing in

228 these frequencies is not impaired by DBS. Nonetheless, several caveats must be taken into

229 account when interpreting ABR thresholds. Although common frequencies were tested (10, 16,

230 24 and 32 kHz) in these studies, hearing loss can occur in other specific frequency bands. In rats,

231 hearing thresholds based on ABRs tend to be at least 10-20 dB higher than those determined

232 behaviorally (Borg, 1982; Heffner et al., 1994). The thresholds in the current study (ranging from

233 36 to 46 dB peSPL) are thus an overestimation of the actual hearing level. To get the most

234 reproducible ABR data in various measurements, we implanted ABR electrodes. In contrast to

235 the commonly used subcutaneous electrodes, these implanted electrodes always measure from

236 exactly the same anatomical position (Buchwald et al., 1981; Hall, 1990; McGee, Ozdamar and

237 Kraus, 1983). To our knowledge, no other studies determined ABR thresholds during HFS and

238 LFS of the CIC or DCBN. Likewise, determination of thresholds in ablation studies, whose

239 results are thought to be similar to HFS, have not been performed.

240 *ABR latency*

241 In addition to thresholds, the latency and amplitude can be extracted from the five ABR peaks.

242 Interpeak latencies are generally accepted as measures of conduction time of the central auditory

243   pathway (Eggermont and Don, 1986; Picton et al., 1977; Squires, Chu and Starr, 1978). The

244   interpeak latency of waves I-III, III-V and I-V reflect the time to traverse in the caudal, rostral

245   and the whole brainstem, respectively. A prolonged interpeak latency reflects a lesion in central

246   auditory processing (Burkhard, Eggermont and Don, 2007; Hood, 1998). Occasionally, a

247   decreased latency of peak V was noted in ablation studies of the IC. This decrease of peak V

248   latency was only an acute effect (Achor and Starr, 1980).

249   In this study, no statistically significant differences were found between the interpeak latencies at

250   baseline compared to low and high frequency DBS. This can be interpreted as no functional

251   relevant lesion at the IC is induced by DBS. However, many studies found no differences in

252   latencies when ablating the IC, but found a difference in amplitude (Achor and Starr, 1980;

253   Buchwald and Huang, 1975; Caird and Klinke, 1987). Therefore, we also performed analysis of

254   the ABR amplitude.

255   *ABR Amplitude*

256   Synchronously activated neurons contribute to the amplitude of the waveform (Burkhard,

257   Eggermont and Don, 2007). The IC has a central role in the auditory pathway (Aitkin and

258   Moore, 1975; De Martino et al., 2013). Previous studies have shown that lesioning of the IC

259   resulted in a decrease of the amplitude of peak V (Achor and Starr, 1980; Buchwald and Huang,

260   1975; Caird and Klinke, 1987). In most studies a large part or the whole IC was ablated. One

261   study only found an abolished peak V when ablation of the lateroventral part of the IC, in

262   contrast to ablating the central nucleus (Funai and Funasaka, 1983). In humans, absence of the IC

263   also resulted in abolished peak V peaks (Durrant et al., 1994). It is assumed that electrode

264   implantation does not influence the amplitude of the evoked potentials, since only minimal tissue

265   damage is seen along the electrode trajectory (Tan et al., 2010).

266   Although the precise role of the cerebellum and its associated nuclei in hearing is not known, it

267   might have a modulatory effect on hearing. The cerebellum receives direct connections from the

268   cochlear nucleus (Huang, Liu and Huang, 1982) and indirect connections from the IC (Aitkin

269   and Boyd, 1978; Huffman and Henson, 1990). Furthermore, auditory stimuli as well as

270   stimulation of the auditory cortex elicited responses from auditory cells in the paraflocculus

271   (Azizi, Burne and Woodward, 1985).

272   One study assessed the ABR during cerebellar stimulation. High frequency stimulation (400 Hz)

273   of the cerebellar surface resulted in a difference of the IV/I amplitude ratio, where peak IV

274   represented in this particular study the IC. The IV/I amplitude ratio increased in case of a short

275   electrical-sound stimulus interval (< 10 ms), and decreased with larger intervals (> 10 ms). In

276   this particular study, peak IV represented the IC (Crispino and Bullock, 1984). In our study, the

277   electrical and sound stimuli were played in an asynchronous manner and therefore various

278   interval times are achieved. This could explain why we did not found any difference in the

279   amplitude ratio. As far as we know, no ABRs were recorded in a cerebellar ablation study.

280   *General ABR findings*

281   It is a well-known phenomenon that high frequency tones show shorter latency peaks than lower

282   frequency sounds, because high frequency sounds stimulate the more basal portions of the basilar

283   membrane (Alvarado et al., 2012). This is also seen in our data. We also found that the peak V

284   amplitude ratio decreased with increasing frequency of the tone given. As far as we know this is

285   a new finding, which has not been reported earlier.

286 *Mechanism of DBS in the auditory system*

287 Our results show that latencies were not prolonged and amplitudes were not decreased during

288 DBS, indicating that DBS in the CIC and DCBN probably does not have an overall inhibitory

289 effect on physiological central auditory processing up to the inferior colliculus (peak V). This

290 finding is supported by one of the main working mechanisms of DBS. Namely that DBS with

291 frequencies above 100 Hz disrupts abnormal information flow in a network (Chiken and Nambu,

292 2014), without influencing the normal neurophysiological activity.

293 HFS is also often referred to as having an inhibitory effect and thus mimicking the effect of a

294 lesion (Benabid et al., 1998; Dostrovsky and Lozano, 2002). The pathological neural network

295 loop related to tinnitus is interrupted by performing HFS within this loop (Smit et al., 2016). This

296 hypothesis is supported by the disruption theory; DBS can dissociate the input and output in a

297 stimulation nucleus and thereby disrupting abnormal information flow such as increased burst

298 activity. Physiological information can still be normally processed through different nuclei

299 (Chiken and Nambu, 2014). It can be hypothesized that this is the same when DBS is applied in

300 the auditory pathway and physiological auditory information processing remains intact.

301 *Future studies*

302 In the current study animal did not receive noise trauma for induction of tinnitus. We

303 hypothesize that if DBS does not result in hearing loss in the normal hearing, this will also not be

304 the case when there is hearing loss in association with tinnitus. The current stimulation

305 parameters can be used for tinnitus treatment; in a recent study that showed a decrease of tinnitus

306 during IC stimulation (Smit et al., 2016), the same stimulation parameters were used as in the

307 current study. In our study no pre-operative assessment of the ABR was performed.

308 *Conclusions*

309 In conclusion, HFS and LFS in the CIC and DCBN did not result in increased ABR thresholds

310 and changes in interpeak latencies. Based on these observations no evidence for changes in

311 information processing in the auditory circuit were found during low and high frequency DBS in

312 the CIC and DCBN. These findings suggest that DBS in the auditory pathways can be performed

313 without hampering physiological processing of auditory information.

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434

435 **Appendix: Absolute values of latencies and amplitudes**

436

437 **Table 1**: Absolute values of latencies and amplitudes from the five peaks of the auditory

438 brainstem response of 10 kHz auditory stimuli. Mean values with standard deviation are given.

Peak	Wave	CIC group			DCBN group		
		Stim-off	**LFS**	**HFS**	**Stim-off**	**LFS**	**HFS**
Latencies	I	1.639 (.073)	1.599 (.110)	1.618 (.080)	1.540 (.165)	1.490 (.076)	1.570 (.090)
	II	2.460 (.092)	2.479 (.121)	2.392 (.079)	2.386 (.162)	2.325 (.066)	2.356 (.083)
	III	3.143 (.181)	3.122 (.208)	3.088 (.144)	3.060 (.280)	3.080 (.109)	3.101 (.084)
	IV	4.288 (.281)	4.167 (.191)	4.122 (.238)	4.077 (.260)	4.118 (.165)	4.148 (.098)
	V	5.050 (.275)	5.047 (.180)	4.765 (.291)	4.903 (.270)	4.923 (.206)	4.983 (.015)
Amplitudes	I	.007 (.018)	.028 (.030)	.037 (.042)	.030 (.016)	.030 (.011)	.041 (.047)
	II	.221 (.018)	.218 (.042)	.252 (.054)	.159 (.012)	.181 (.009)	.185 (.048)
	III	.042 (.018)	.074 (.037)	.076 (.038)	.078 (.031)	.097 (.066)	.117 (.069)
	IV	.070 (.017)	.086 (.042)	.117 (.035)	.061 (.055)	.087 (.041)	.103 (.053)
	V	.030 (.059)	.054 (.049)	.063 (.043)	.077 (.028)	.069 (.023)	.093 (.039)

439   Abbreviations: CIC = central nucleus of interior colliculus, DCBN = dentate cerebellar nucleus,

440   stim-off = stimulation-off state, LFS = deep brain stimulation at 10 Hz, HFS = deep brain

441   stimulation at 100 Hz.

442   **Table 2**: Absolute values of latencies and amplitudes from the five peaks of the auditory

443   brainstem response of 16 kHz auditory stimuli. Mean values with standard deviation are given.

Peak	Wave	CIC group			DCBN group		
		Stim-off	LFS	HFS	Stim-off	LFS	HFS
Latencies	I	1.400 (.153)	1.419 (.042)	1.530 (.076)	1.399 (.042)	1.389 (.027)	1.470 (.066)
	II	2.245 (.153)	2.275 (.055)	2.325 (.075)	2.215 (.128)	2.285 (.084)	2.275 (.065)
	III	2.960 (.292)	2.960 (.125)	3.020 (.155)	2.96 (.140)	2.929 (.075)	3.030 (.090)
	IV	3.956 (.367)	4.067 (.190)	4.057 (.230)	3.977 (.118)	4.027 (.155)	4.098 (.145)
	V	4.782 (.534)	4.903 (.206)	4.883 (.249)	4.822 (.240)	4.863 (.213)	4.993 (.120)
Amplitudes	I	.042 (.040)	.039 (.031)	.037 (.027)	.036 (.018)	.025 (.016)	.058 (.046)
	II	.182 (.028)	.162 (.030)	.189 (.043)	.139 (.052)	.119 (.018)	.178 (.023)
	III	.079 (.048)	.080 (.048)	.085 (.029)	.088 (.047)	.079 (.040)	.118 (.052)
	IV	.050 (.019)	.057 (.031)	.089 (.029)	.047 (.015)	.072 (.030)	.113 (.035)
	V	.067 (.047)	.050 (.028)	.077 (.029)	.080 (.089)	.034 (.023)	.086 (.040)

444   Abbreviations: CIC = central nucleus of interior colliculus, DCBN = dentate cerebellar nucleus,

445   stim-off = stimulation-off state, LFS = deep brain stimulation at 10 Hz, HFS = deep brain

446   stimulation at 100 Hz.

447

448   **Table 3**: Absolute values of latencies and amplitudes from the five peaks of the auditory

449   brainstem response of 24 kHz auditory stimuli. Mean values with standard deviation are given.

Peak	Wave	CIC group			DCBN group		
		Stim-off	**LFS**	**HFS**	**Stim-off**	**LFS**	**HFS**
Latencies	I	1.379 (.126)	1.379 (.027)	1.480 (.131)	1.299 (.083)	1.399 (.075)	1.419 (.066)
	II	2.235 (.200)	2.215 (.071)	2.293 (.111)	2.134 (.116)	2.225 (.083)	2.305 (.066)
	III	2.929 (.234)	2.929 (.114)	3.050 (.131)	2.859 (.140)	2.919 (.094)	3.040 (.121)
	IV	3.896 (.326)	3.987 (.186)	4.097 (.272)	3.906 (.170)	4.007 (.116)	4.108 (.166)
	V	4.782 (.391)	4.933 (.228)	5.023 (.262)	4.570 (.215)	4.812 (.275)	4.790 (.129)
Amplitudes	I	.026 (.025)	.023 (.038)	.033 (.027)	.032 (.011)	.028 (.014)	.046 (.045)
	II	.116 (.020)	.094 (.020)	.110 (.028)	.090 (.016)	.088 (.015)	.117 (.029)
	III	.080 (.062)	.072 (.062)	.074 (.031)	.070 (.033)	.055 (.034)	.085 (.072)
	IV	.037 (.033)	.192 (.301)	.083 (.054)	.053 (.024)	.054 (.009)	.077 (.041)
	V	.034 (.036)	.033 (.045)	.043 (.028)	.022 (.014)	.013 (.013)	.050 (.039)

450   Abbreviations: CIC = central nucleus of interior colliculus, DCBN = dentate cerebellar nucleus,

451   stim-off = stimulation-off state, LFS = deep brain stimulation at 10 Hz, HFS = deep brain

452   stimulation at 100 Hz.

453

454   **Table 4**: Absolute values of latencies and amplitudes from the five peaks of the auditory

455   brainstem response of 32 kHz auditory stimuli. Mean values with standard deviation are given.

Peak	Wave	CIC group			DCBN group		
		Stim-off	**LFS**	**HFS**	**Stim-off**	**LFS**	**HFS**
Latencies	I	1.369 (.826)	1.408 (.051)	1.497 (.188)	1.289 (.104)	1.268 (.042)	1.409 (.155)
	II	2.235 (.145)	2.222 (.106)	2.272 (.122)	2.104 (.180)	1.980 (.444)	2.325 (.153)
	III	2.919 (.155)	2.885 (.124)	2.926 (.139)	2.859 (1.80)	2.789 (.098)	2.980 (.199)
	IV	3.946 (.210)	3.912 (.130)	3.982 (.207)	3.866 (.232)	4.097 (.614)	4.027 (.216)
	V	4.802 (.413)	4.896 (.371)	4.776 (.304)	4.681 (.222)	4.691 (.271)	4.842 (.277)
Amplitudes	I	.017 (.011)	.024 (.032)	.052 (.124)	.035 (.017)	.023 (.013)	.043 (.041)
	II	.097 (.019)	.156 (.150)	.167 (.225)	.078 (.021)	.091 (.030)	.092 (.030)
	III	.062 (.015)	.093 (.091)	.134 (.145)	.058 (.031)	.056 (.063)	.058 (.040)
	IV	.057 (.048)	.129 (.141)	.190 (.268)	.034 (.026)	.048 (.028)	.066 (.042)
	V	.021 (.022)	.032 (.054)	.039 (.090)	.015 (.146)	.013 (.025)	0.042 (.043)

456   Abbreviations: CIC = central nucleus of interior colliculus, DCBN = dentate cerebellar nucleus,

457   stim-off = stimulation-off state, LFS = deep brain stimulation at 10 Hz, HFS = deep brain

458   stimulation at 100 Hz.

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Interpeak latencies (IL) and V/I amplitude ratio (AR) for 10k, 16k, 24k and 32k burst sounds. Mean values with standard deviation are given. Adjusted Holm-Bonferroni *p*-values are used.

Frequency (Hz)	Peaks	CIC group					DCBN group				
		Stim-off	LFS	*p*	HFS	*p*	Stim-off	LFS	*p*	HFS	*p*
10k	IL I–III	1.504 (.170)	1.524 (.125)	> .99	1.470 (.285)	> .99	1.520 (.157)	1.590 (.162)	0.20	1.530 (.136)	> .99
	IL III–V	1.908 (.213)	1.925 (.128)	> .99	1.745 (.285)	> .99	1.842 (.028)	1.842 (.116)	> .99	1.953 (.109)	.32
	IL I–V	3.411 (.312)	3.449 (.143)	> .99	3.24 (.291)	> .99	3.362 (.153)	3.433 (.245)	> .99	3.514 (.130)	.22
	AR V/I	1.542 (1.739)	1.238 (.832)	> .99	1.174 (.517)	> .99	1.349 (.946)	1.1310 (.398)	> .99	1.326 (.287)	> .99
16k	IL I–III	1.560 (.160)	1.540 (.131)	> .99	1.490 (.145)	0.25	1.560 (.147)	1.540 (.084)	> .99	1.561 (.062)	> .99
	IL III–V	1.822 (.257)	1.943 (.091)	> .99	1.862 (.113)	> .99	1.863 (.113)	1.933 (.166)	.85	1.9630 (.050)	> .99
	IL I–V	3.383 (.395)	3.483 (.218)	> .99	3.353 (.248)	> .99	3.423 (.252)	3.474 (.214)	> .99	3.5236 (.094)	> .99
	AR V/I	.979 (.410)	.9067 (.320)	.69	1.310 (.410)	> .99	1.178 (.913)	1.278 (1.470)	> .99	1.078 (.289)	> .99

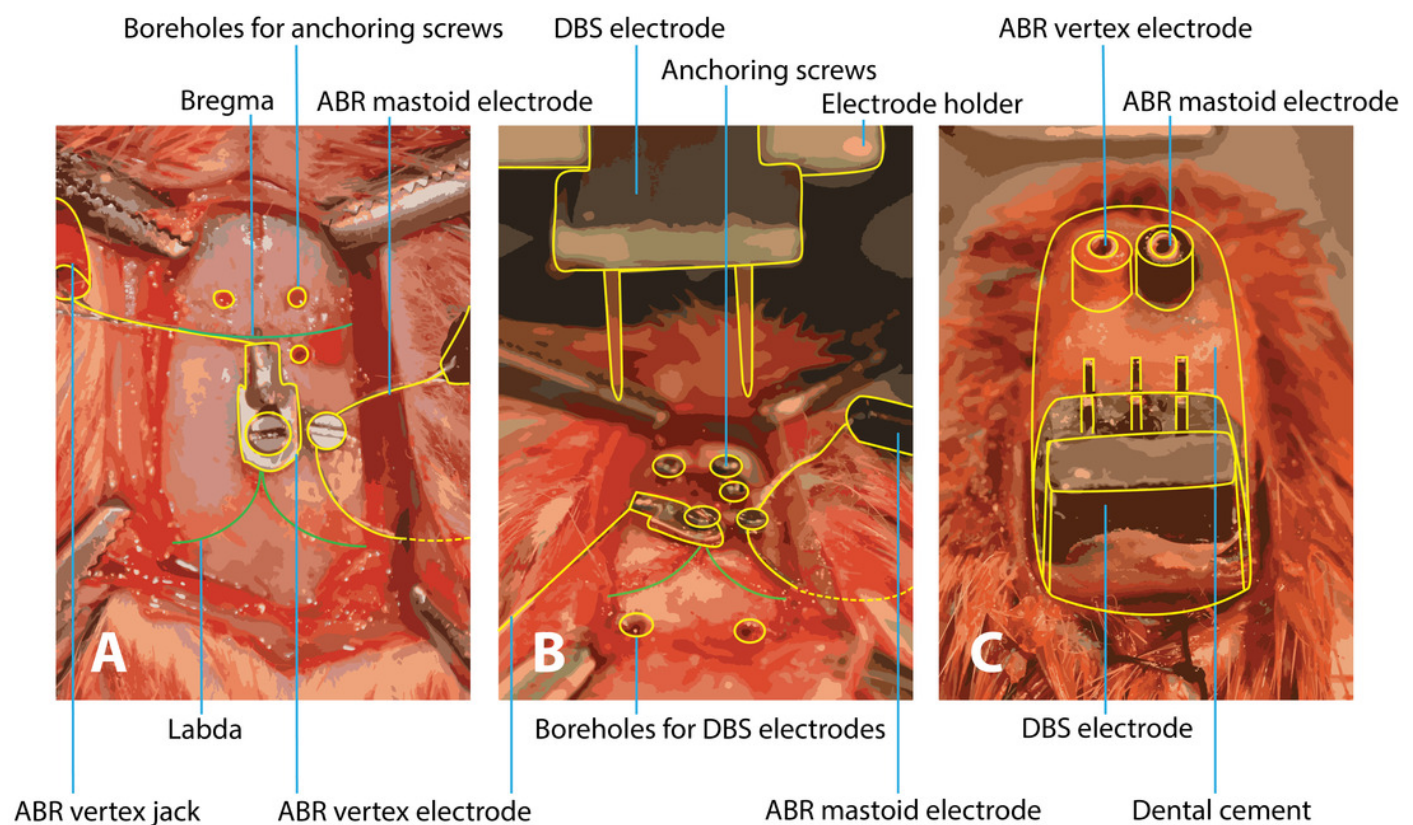
24k	IL I-III	1.550 (.157)	1.550 (.130)	> .99	1.570 (.232)	> .99	1.560 (.113)	1.520 (.090)	> .99	1.621 (.120)	> .99
	IL III-V	1.853 (.186)	2.004 (.135)	0.25	1.974 (.180)	> .99	1.712 (.202)	1.893 (.293)	.25	1.750 (.200)	> .99
	IL I-V	3.403 (.329)	3.554 (.243)	0.26	3.544 (.309)	> .99	3.272 (.250)	3.413 (.279)	> .99	3.371 (.147)	.51
	AR V/I	.957 (.242)	.916 (.594)	> .99	1.11 (.595)	> .99	.536 (.265)	.445 (.305)	.50	.834 (.470)	.50
32k	IL I-III	1.550 (.109)	1.478 (.093)	> .99	1.428 (.261)	> .99	1.570 (.097)	1.520 (.066)	.23	1.570 (.157)	> .99
	IL III-V	1.883 (.318)	2.010 (.376)	> .99	1.850 (.240)	> .99	1.822 (.065)	1.903 (.272)	> .99	1.862 (.366)	> .99
	IL I-V	3.433 (.400)	3.488 (.372)	> .99	3.278 (.416)	> .99	3.393 (.145)	3.423 (.283)	> .99	3.433 (.362)	> .99
	AR V/I	.491 (.437)	.505 (.278)	.69	.617 (.523)	.28	.404 (.296)	.551 (.209)	.50	.870 (.961)	.45

2   Abbreviations: CIC = central nucleus of interior colliculus, DCBN = dentate cerebellar nucleus, stim-off = stimulation-off state, LFS =

3   deep brain stimulation at 10 Hz, HFS = deep brain stimulation at 100 Hz.

Figure 1

Surgery of implantation of ABR and DBS electrodes.

A. After exposing the skull, the vertex electrode is attached with a screw in the skull and the mastoid electrode is subcutaneously tunneled to the mastoid and also fixated with a screw. Three boreholes are made for anchoring screws to later fixate the structure with dental cement. B. Boreholes for the DBS electrodes are drilled at coordinates calculated from the bregma level. Calculation of the boreholes and placement of the DBS electrodes are performed within a stereotactic frame. C. All electrodes are in place and the construct is fixated with dental cement.

ABR signal processing

A. Example of a raw signal that was measured during low frequency stimulation (LFS). B. Stimulation artifacts are filtered with automatic peak detection analysis. C. Example of an auditory brainstem response (ABR) (burst frequency 10 kHz) during off-stimulation state, during LFS and during high frequency stimulation (HFS) in the central nucleus of the inferior colliculus (CIC). The five ABR peaks are numbered I-V. Morphology and latency of ABR peaks in the current study were consistent with other animal studies (Backoff and Caspary, 1994; Dehmel et al., 2012; Zheng et al., 2012). The first peak arises approximately 1.5 ms after stimulus onset. Although there is overlap, the first peak represents neural activity of the cochlear nerve. The second peak is considered to be mainly generated by cochlear nuclear cells, the third peak by the contralateral superior olivary complex cells and the fourth peak by the lateral lemniscus. The fifth peak, which appears approximately 5 ms after onset, originates from the IC (Biacabe et al., 2001; Chen and Chen, 1991; Simpson et al., 1985).

Raw signal recordings during LFS

A

B

Unfiltered recordings

Filtered with automatic peak analysis

Auditory Brainstem Response

Off-stimulation state

LFS

HFS

C

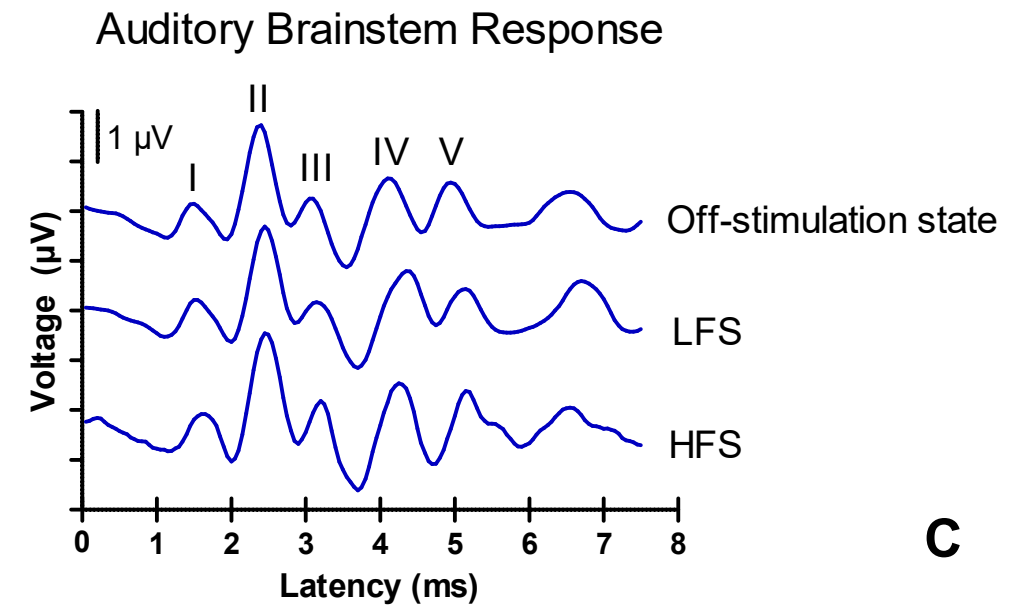
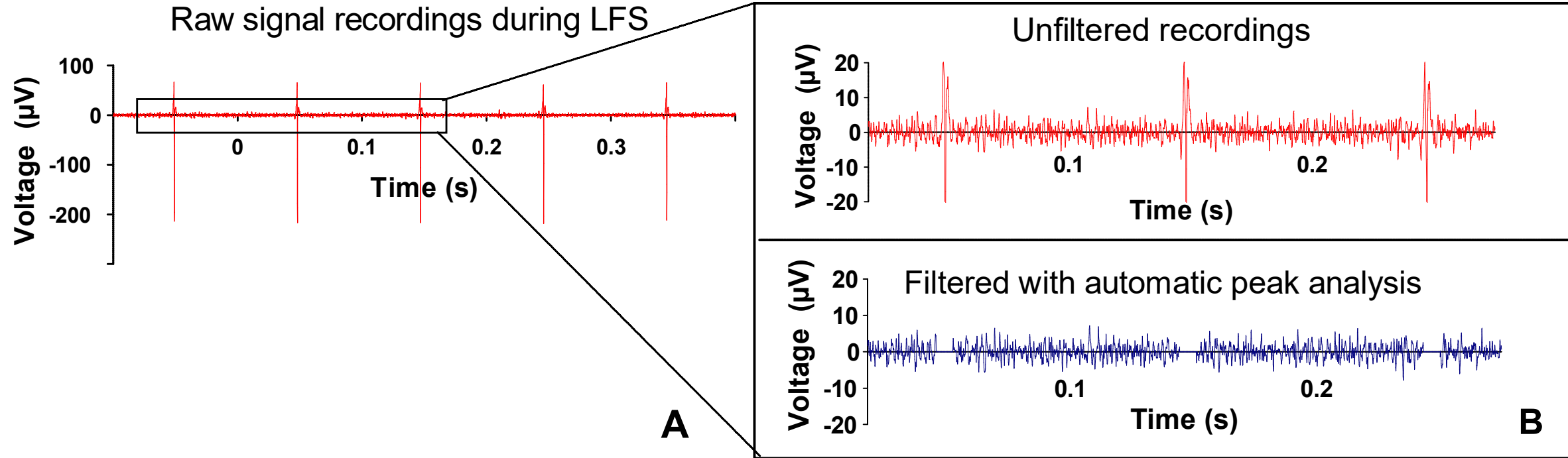
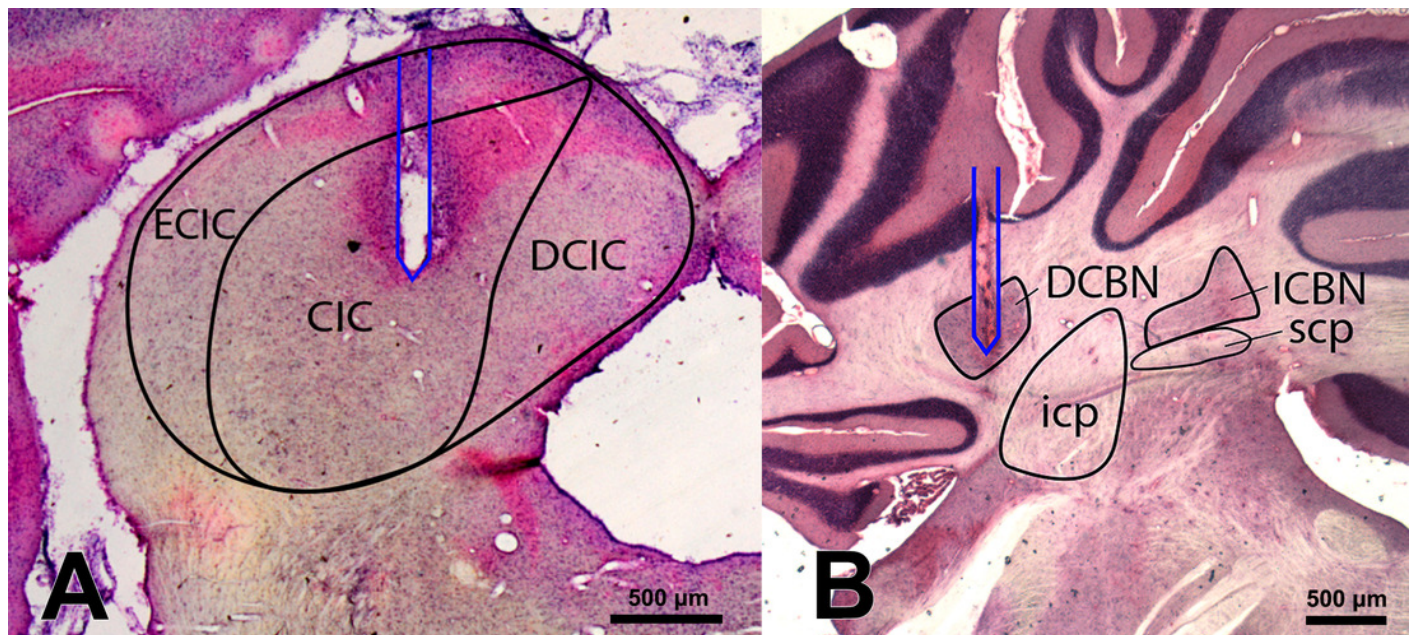



Figure 3

Histology

Representative examples of electrode positions (white lines) in the CIC (A) and DCBN (B). All electrodes were implanted bilaterally. ECIC = external nucleus of the inferior colliculus, CIC = central nucleus of inferior colliculus, DCIC = dorsal cortex of inferior colliculus, DCBN = dentate cerebellar nucleus, icp = inferior cerebellar peduncle, ICBN = interposed cerebellar nucleus, scp = superior cerebellar peduncle. Scale bar: 500 μm.

ABR thresholds

ABR thresholds of the CIC (A) and DCBN group (B) measured during the DBS-off state (blue, circles, solid line), LFS (green, squares, striped line) and HFS (red, triangles, dotted line). There was no statistically significant difference. The vertical lines indicate the standard error of the mean.