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

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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.
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Abstract

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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.

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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
external nucleus of the inferior colliculus (IC) in rats decreased tinnitus-like behavior (Smit et al., 2016). The cerebellum is a structure that is not involved in the auditory pathways but is associated with tinnitus (Brozoski, Ciobanu and Bauer, 2007; Osaki et al., 2005; Sedley et al., 2012; Shulman and Strashun, 1999). It was demonstrated that ablation of the paraflocculus completely diminished tinnitus in rats (Bauer et al., 2012). The majority of fibers in the cerebellum, including the paraflocculus, originate from the deep cerebellar nuclei, especially the DCBN, which is the largest (Gayer and Faull, 1988; Gould, 1979). Therefore, the CIC and the DCBN could be considered as respectively an auditory and a non-auditory potential DBS target for the treatment of tinnitus.

DBS can be performed with low frequency stimulation (LFS), which mainly has an excitatory effect, and as HFS, which generally is described as a global inhibitory effect similar as ablation (Benabid et al., 1998; Breit, Schulz and Benabid, 2004; Dostrovsky and Lozano, 2002). Following ablation of IC in animals models, decreased amplitude and latency of peak V have been found (Achor and Starr, 1980; Buchwald and Huang, 1975; Durrant et al., 1994; Kaga, Shinoda and Suzuki, 1997). Peak V is the last of the five peaks of the ABR and represents neural activity of the IC. Because of a high variability in amplitude among subjects, the V/I amplitude ratio is a more consistent measure than the absolute value (Musiek et al., 1984; Musiek, Reeves and Baran, 1985). The relative measures of the latencies are the interpeak latencies (I-III, III-V, I-V) which represent the central transmission latency best (Eggermont and Don, 1986; Picton et al., 1977; Squires, Chu and Starr, 1978). There is little evidence that stimulation of cerebellar structures has influence on the ABR (Crispino and Bullock, 1984).

We hypothesize that for CIC stimulation, the V/I amplitude ratio of the ABR would be lower and the I-V or III-V interpeak latencies would be prolonged during HFS and not during LFS of the
Our hypothesis is that stimulating a non-auditory structure such as the DCBN does not have any influence on the ABR.
Methods

Animals

Male rats (Sprague Dawley, 250-300 g, Charles River, The Netherlands) were housed individually under conditions of constant room temperature and humidity with a reversed 12u/12u light/dark cycle and had free access to water and food. The Animal Experiments Committee of the Maastricht University approved the experiments (approval reference number 2012-069).

Surgical procedure

Subcutaneous electrodes were implanted for ABR recordings and during the same surgery DBS electrodes were implanted in the brain (Figure 1). Animals were anesthetized by intraperitoneal administration of ketamine (90 mg/kg) and xylazine (10 mg/kg). The head of the rats was immobilized in a stereotactic apparatus (Stoelting Co, Wood Dale, Illinois) with mouth and blunt ear-bars. Permanent Teflon-coated stainless steel electrodes were subcutaneously implanted. One wire electrode was subcutaneously tunneled to the mastoid and a second wire electrode was attached to a screw on the vertex. Based on coordinates from a stereotactic atlas (Paxinos and Watson, 2007), bilateral electrodes (Technomed, Beek, The Netherlands) were inserted in the CIC (bregma -8.8, depth 4.5, interspace 3.8) or in the DCBN (bregma -11.5, depth 6.5, interspace 6.8). The postoperative recovery time was one week.

Deep brain stimulation

DBS was performed with bipolar, concentric electrodes using monophasic rectangular pulses. The electrical stimulus pulses were created by an A310 accupulser and an A360 stimulus isolator.
(World Precision Instruments, Berlin, Germany). During DBS, stimuli were given with a frequency of 100 Hz (HFS) and 10 Hz (LFS) with an amplitude of 100 µA and a pulse width of 60 µs. Electrodes are gold-plated with platinum-iridium inner wire (negative contact) and stainless steel outer part (positive contact). The inner and outer electrodes are insulated except for a 75 µm exposed tip (Tan et al., 2010).

Rats were divided in two groups, one group received implantation of electrodes in the CIC (n=5) and the other group in the DCBN (n=5). In the off-stimulation state, designated as the control situation, no electrical stimulation was given. During stimulation-off state, LFS (10 Hz) and HFS (100 Hz), ABRs were recorded in separate sessions.

**Auditory brainstem response**

ABR measurements were performed in a random manner of the three situations (off-stimulation, LFS, HFS) with a one week interval. Stimulation was turned on approximately 5 minutes before ABR recordings. HFS consisted of a concentric bipolar electrode using monophasic rectangular pulses, with a frequency of 100 Hz, amplitude of 100 µA per electrode and a pulse width of 60 µs (A310 Acupulser, World Precision Instruments, Berlin, Germany). Similar settings were used in a study which showed tinnitus reduction during HFS in rats (Smit et al., 2016). LFS consisted of the same parameters with a frequency of 10 Hz.

To achieve anesthesia during ABR recordings, intraperitoneal administration of ketamine (90 mg/kg) and xylazine (10 mg/kg) was used, which is preferred over isoflurane when assessing hearing thresholds in rats (Ruebhausen, Brozoski and Bauer, 2012).
During the ABR procedure, animals were placed into a sound-attenuating chamber. Cables were plugged into the socket of the head of the animal and connected to the recording device (Powerlab 8/35 connected to a Dual Bio Amp amplifier (ADInstruments, Castle Hill, Australia). An electrode connected to the left hind paw served as the ground.

Custrom-made auditory stimuli (10, 16, 24 and 32 kHz) were created with Matlab 2011a (Mathworks, MA, USA) and consisted of 5 ms bursts with a \( \cos^2 \) rise and fall filter and were played at a rate of 20 per second at decreasing intensities from 90 to 0 dB peSPL with steps of 10 dB. To prevent synchronous occurrence of stimulation artifacts with the ABRs, one in 10 stimuli had an interval of 55 ms instead of 45 ms. To gain an approximately similar amount of data after filtering of stimulation artefacts, 500 auditory stimuli were given per intensity in the off-stimulation state, 700 during LFS and 1000 during HFS. Sounds were calibrated with a Bruel & Kjaer 2231 decibel meter with a 4191 microphone (range 2-40 kHz), which was placed at the location of the rat’s right ear. Sound intensities are reported as the peak equivalent sound pressure level (peSPL).

Auditory stimuli were processed with an external soundcard with a sample rate of 192 kHz (Creative E-MU 0204), amplified with Ultrasonic power amplifier (Avisoft Bioacoustics, Berlin) and played with an Ultrasonic Dynamic Speaker Vifa (Avisoft Bioacoustics, Berlin, Germany) to the right ear. To standardize sound presentation between recording sessions it was monitored that in every session the same position of the rat and the same distance between the loudspeaker and the ear was used (2 cm). The contralateral ear was plugged with modeling clay.

Auditory stimuli were digitally triggered. The recordings were done in Labchart Pro 7 (ADInstruments, Castle Hill, Australia) at a sample frequency of 20 kHz and raw data were
imported into Matlab. With a customized script, the signal was amplified 100,000 times and
band-pass filtered (300-3000 Hz). Evoked responses were averaged and data which contained
DBS artifacts were automatically removed based on a peak-detection analysis. Using a
customized Matlab script, peaks were automatically detected if the signal was above a manual
depicted maximal baseline value. Before and after the maximal value of the peak of the artefact
2.5ms of data were converted in Not-a-Number (NaN). The ABR and DBS stimuli were not
phase-locked so per epoch a different part was converted in NaN. All epochs were averaged to
calculate the mean ABR signal (Figure 2B).

Two independent blinded observers visually identified ABR thresholds and peaks. In case of
disagreement, a third observer was sought and the concordant data were accepted. The auditory
threshold was defined as the lowest decibel level (peSPL) of the stimulus, which produced a
distinctive ABR.

For latency analysis, the five positive peaks were determined at 90 dB peSPL and numbered I-V
based on the recordings of vertex upward deflections (for an example see Figure 2C). Latencies
of peaks were measured from stimulus onset. Interpeak latency was defined as the time between
respective peaks.

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

*Electrode localization*

Animals were deeply anesthetized with pentobarbital (75 mg/kg) and perfused transcardially
with Tyrode’s buffer (0.1 M) and fixative containing 4% paraformaldehyde, 15% picric acid and
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
sections containing the target area and the electrode trajectory were stained with hematoxylin-
eosin (Merck, Darmstadt, Germany). Definition of anatomic structures was based on the
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).
Results

Electrode localization

Histological evaluation showed that all electrodes were implanted correctly in the target structures (Figures 3A and 3B, respectively).

Hearing thresholds

Hearing response thresholds were determined as the minimal intensity stimulus at which an ABR was evident. Thresholds of different stimulus frequencies (10, 16, 24 and 32 kHz) are depicted in Figure 4A for the CIC group and in Figure 4B for the DCBN group. In one rat two thresholds (10 Hz LFS and 32 Hz LFS) were not possible to determine. In both groups, no statistically significant differences were found during HFS and LFS compared to off-stimulation.

Latencies and amplitudes

From all ABRs, 5 distinctive peaks could be determined at 90 dB peSPL (Figure 1). In Table 1 the mean interpeak latencies (I-III, III-V and I-V) are shown for different burst frequencies (10, 16, 24 and 32 kHz). In both the CIC and the DCBN group, no statistically significant differences were found for high and low frequency DBS compared to no stimulation (Table 1).

The V/I amplitude ratio was calculated at all burst frequencies. In both groups, there was no statistical significant difference when comparing no stimulation with HFS and LFS. Appendix 1 shows the absolute latencies and interpeak latencies.
When looking at the latency and amplitude data, a relation between ABR latencies and amplitudes, with frequencies of burst tones was noticed. For further analysis, we grouped the off-stimulation data of the CIC and DCBN group since only baseline measurements were analyzed. The latency, e.g. of peak I, differed between burst frequencies ($\chi^2(3) = 20.12, p < 0.01$). The raw data (see Appendix 1) show a shorter latency with increasing frequencies of burst tones. The V/I amplitude ratio does not differ amongst frequencies ($\chi^2(3) = 4.92, p = .178$). Amplitudes of peak I did not differ between frequencies ($\chi^2(3) = 3.240, p = .355$), but the amplitude of peak V was different between frequencies ($\chi^2(3) = 17.160, p < 0.01$). Also peak V amplitude decreases with increasing burst frequency.
We successfully measured ABRs during stimulation-off state, LFS and HFS. Our results showed that LFS as well as HFS in the CIC and DCBN do not influence ABR thresholds, interpeak latencies and amplitude ratios in rats.

**ABR thresholds**

The finding that ABR thresholds were not influenced by LFS and HFS suggests that hearing in these frequencies is not impaired by DBS. Nonetheless, several caveats must be taken into account when interpreting ABR thresholds. Although common frequencies were tested (10, 16, 24 and 32 kHz) in these studies, hearing loss can occur in other specific frequency bands. In rats, hearing thresholds based on ABRs tend to be at least 10-20 dB higher than those determined behaviorally (Borg, 1982; Heffner et al., 1994). The thresholds in the current study (ranging from 36 to 46 dB peSPL) are thus an overestimation of the actual hearing level. To get the most reproducible ABR data in various measurements, we implanted ABR electrodes. In contrast to the commonly used subcutaneous electrodes, these implanted electrodes always measure from exactly the same anatomical position (Buchwald et al., 1981; Hall, 1990; McGee, Ozdamar and Kraus, 1983). To our knowledge, no other studies determined ABR thresholds during HFS and LFS of the CIC or DCBN. Likewise, determination of thresholds in ablation studies, whose results are thought to be similar to HFS, have not been performed.

**ABR latency**

In addition to thresholds, the latency and amplitude can be extracted from the five ABR peaks. Interpeak latencies are generally accepted as measures of conduction time of the central auditory
The interpeak latency of waves I-III, III-V and I-V reflect the time to traverse in the caudal, rostral and the whole brainstem, respectively. A prolonged interpeak latency reflects a lesion in central auditory processing (Burkhard, Eggermont and Don, 2007; Hood, 1998). Occasionally, a decreased latency of peak V was noted in ablation studies of the IC. This decrease of peak V latency was only an acute effect (Achor and Starr, 1980).

In this study, no statistically significant differences were found between the interpeak latencies at baseline compared to low and high frequency DBS. This can be interpreted as no functional relevant lesion at the IC is induced by DBS. However, many studies found no differences in latencies when ablating the IC, but found a difference in amplitude (Achor and Starr, 1980; Buchwald and Huang, 1975; Caird and Klinke, 1987). Therefore, we also performed analysis of the ABR amplitude.

**ABR Amplitude**

Synchronously activated neurons contribute to the amplitude of the waveform (Burkhard, Eggermont and Don, 2007). The IC has a central role in the auditory pathway (Aitkin and Moore, 1975; De Martino et al., 2013). Previous studies have shown that lesioning of the IC resulted in a decrease of the amplitude of peak V (Achor and Starr, 1980; Buchwald and Huang, 1975; Caird and Klinke, 1987). In most studies a large part or the whole IC was ablated. One study only found an abolished peak V when ablation of the lateroventral part of the IC, in contrast to ablating the central nucleus (Funai and Funasaka, 1983). In humans, absence of the IC also resulted in abolished peak V peaks (Durrant et al., 1994). It is assumed that electrode
Implantation does not influence the amplitude of the evoked potentials, since only minimal tissue damage is seen along the electrode trajectory (Tan et al., 2010).

Although the precise role of the cerebellum and its associated nuclei in hearing is not known, it might have a modulatory effect on hearing. The cerebellum receives direct connections from the cochlear nucleus (Huang, Liu and Huang, 1982) and indirect connections from the IC (Aitkin and Boyd, 1978; Huffman and Henson, 1990). Furthermore, auditory stimuli as well as stimulation of the auditory cortex elicited responses from auditory cells in the paraflocculus (Azizi, Burne and Woodward, 1985).

One study assessed the ABR during cerebellar stimulation. High frequency stimulation (400 Hz) of the cerebellar surface resulted in a difference of the IV/I amplitude ratio, where peak IV represented in this particular study the IC. The IV/I amplitude ratio increased in case of a short electrical-sound stimulus interval (< 10 ms), and decreased with larger intervals (> 10 ms). In this particular study, peak IV represented the IC (Crispino and Bullock, 1984). In our study, the electrical and sound stimuli were played in an asynchronous manner and therefore various interval times are achieved. This could explain why we did not found any difference in the amplitude ratio. As far as we know, no ABRs were recorded in a cerebellar ablation study.

**General ABR findings**

It is a well-known phenomenon that high frequency tones show shorter latency peaks than lower frequency sounds, because high frequency sounds stimulate the more basal portions of the basilar membrane (Alvarado et al., 2012). This is also seen in our data. We also found that the peak V amplitude ratio decreased with increasing frequency of the tone given. As far as we know this is a new finding, which has not been reported earlier.
Mechanism of DBS in the auditory system

Our results show that latencies were not prolonged and amplitudes were not decreased during DBS, indicating that DBS in the CIC and DCBN probably does not have an overall inhibitory effect on physiological central auditory processing up to the inferior colliculus (peak V). This finding is supported by one of the main working mechanisms of DBS. Namely that DBS with frequencies above 100 Hz disrupts abnormal information flow in a network (Chiken and Nambu, 2014), without influencing the normal neurophysiological activity.

HFS is also often referred to as having an inhibitory effect and thus mimicking the effect of a lesion (Benabid et al., 1998; Dostrovsky and Lozano, 2002). The pathological neural network loop related to tinnitus is interrupted by performing HFS within this loop (Smit et al., 2016). This hypothesis is supported by the disruption theory; DBS can dissociate the input and output in a stimulation nucleus and thereby disrupting abnormal information flow such as increased burst activity. Physiological information can still be normally processed through different nuclei (Chiken and Nambu, 2014). It can be hypothesized that this is the same when DBS is applied in the auditory pathway and physiological auditory information processing remains intact.

Future studies

In the current study animal did not receive noise trauma for induction of tinnitus. We hypothesize that if DBS does not result in hearing loss in the normal hearing, this will also not be the case when there is hearing loss in association with tinnitus. The current stimulation parameters can be used for tinnitus treatment; in a recent study that showed a decrease of tinnitus during IC stimulation (Smit et al., 2016), the same stimulation parameters were used as in the current study. In our study no pre-operative assessment of the ABR was performed.
Conclusions

In conclusion, HFS and LFS in the CIC and DCBN did not result in increased ABR thresholds and changes in interpeak latencies. Based on these observations no evidence for changes in information processing in the auditory circuit were found during low and high frequency DBS in the CIC and DCBN. These findings suggest that DBS in the auditory pathways can be performed without hampering physiological processing of auditory information.
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Appendix: Absolute values of latencies and amplitudes

Table 1: Absolute values of latencies and amplitudes from the five peaks of the auditory 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) |
Abbreviations: CIC = central nucleus of interior colliculus, DCBN = dentate cerebellar nucleus, stim-off = stimulation-off state, LFS = deep brain stimulation at 10 Hz, HFS = deep brain stimulation at 100 Hz.

Table 2: Absolute values of latencies and amplitudes from the five peaks of the auditory 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 |
| 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) |
Abbreviations: CIC = central nucleus of interior colliculus, DCBN = dentate cerebellar nucleus, stim-off = stimulation-off state, LFS = deep brain stimulation at 10 Hz, HFS = deep brain stimulation at 100 Hz.
Table 3: Absolute values of latencies and amplitudes from the five peaks of the auditory 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 |
|      | 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) |
|      |      | .026 (.025) | .023 (.038) | .033 (.027) | .032 (.011) | .028 (.014) | .046 (.045) |
|      | I    | .116 (.020) | .094 (.020) | .110 (.028) | .090 (.016) | .088 (.015) | .117 (.029) |
|      | II   | .080 (.062) | .072 (.062) | .074 (.031) | .070 (.033) | .055 (.034) | .085 (.072) |
|      | III  | .037 (.033) | .192 (.301) | .083 (.054) | .053 (.024) | .054 (.009) | .077 (.041) |
|      | IV   | .034 (.036) | .033 (.045) | .043 (.028) | .022 (.014) | .013 (.013) | .050 (.039) |

Abbreviations: CIC = central nucleus of interior colliculus, DCBN = dentate cerebellar nucleus, stim-off = stimulation-off state, LFS = deep brain stimulation at 10 Hz, HFS = deep brain stimulation at 100 Hz.
Table 4: Absolute values of latencies and amplitudes from the five peaks of the auditory 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 (.180) | 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) | .042 (.043) |

Abbreviations: CIC = central nucleus of interior colliculus, DCBN = dentate cerebellar nucleus, stim-off = stimulation-off state, LFS = deep brain stimulation at 10 Hz, HFS = deep brain stimulation at 100 Hz.
Table 1 (on next page)

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 (1.157) | 1.550 (1.130) | > .99 | 1.570 (1.232) | > .99 | 1.560 (1.113) | > .99 | 1.520 (0.90) | > .99 | 1.621 (1.120) | > .99 |
|-----|----------|----------------|----------------|-------|---------------|-------|---------------|-------|---------------|-------|---------------|-------|
|     | IL III-V | 1.853 (1.186)  | 2.004 (1.135)  | 0.25  | 1.974 (1.180) | > .99 | 1.712 (1.202) | > .99 | 1.893 (1.293) | .25   | 1.750 (1.200) | > .99 |
|     | IL I-V   | 3.403 (.329)   | 3.554 (.243)   | 0.26  | 3.544 (.309)  | > .99 | 3.272 (.250)  | > .99 | 3.413 (.379)  | > .99 | 3.371 (.147)  | .51   |
|     | AR V/I   | .957 (.242)     | .916 (.594)     | > .99 | 1.11 (.595)   | > .99 | .536 (.265)   | > .99 | .445 (.305)   | .50   | .834 (.470)   | .50   |
| 32k | IL I-III | 1.550 (.109)    | 1.478 (.093)    | > .99 | 1.428 (.261)  | > .99 | 1.570 (.097)  | > .99 | 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)  | > .99 | 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)  | > .99 | 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 = 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.
**Figure 2** (on next page)

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).
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.
Figure 4 (on next page)

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.
