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Auditory brainstem stimulation with a conformable microfabricated array elicits responses with tonotopically organized components

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Abstract

Auditory brainstem implants (ABIs) restore hearing to deaf individuals not eligible for cochlear implants. Speech comprehension in ABI users is generally poor compared to that of cochlear implant users, and side effects are common. The poor performance may result from activating broad areas and multiple neuronal populations of the cochlear nucleus, however detailed studies of the responses to surface stimulation of the cochlear nucleus are lacking. A conformable electrode array was microfabricated to fit on the rat's dorsal cochlear nucleus (DCN). It hosts 20 small electrodes (each 100 μm diam.). The array was tested by recording evoked potentials and neural activity along the tonotopic axis of the inferior colliculus (IC). Almost all bipolar electrode pairs elicited responses, in some cases with an even, or relatively constant, pattern of thresholds and supra-threshold measures along the long axis of the array. This pattern suggests that conformable arrays can provide relatively constant excitation along the surface of the DCN and thus might decrease the ABI side effects caused by spread of high current to adjacent structures. We also examined tonotopic patterns of the IC responses. Compared to sound-evoked responses, electrically-evoked response mappings had less tonotopic organization and were broader in width. They became more tonotopic when the evoked activity common to all electrodes and the late phase of response were subtracted out, perhaps because the remaining activity is from tonotopically organized principal cells of the DCN. Responses became less tonotopic when inter-electrode distance was increased from 400 μm to 800 μm but were relatively unaffected by changing to monopolar stimulation. The results illustrate the challenges of using a surface array to present tonotopic cues and improve speech comprehension in humans who use the ABI.

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Declarations of interest

None.

Keywords

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1. Introduction

The auditory brainstem implant (ABI) is a neuroprosthesis that provides sound sensations by stimulating neurons of the cochlear nucleus (CN) in deaf patients who are not candidates for a cochlear implant (CI). The ABI has been approved by the Food and Drug Administration for adults with Neurofibromatosis Type 2 (NF2), a devastating genetic syndrome associated with bilateral vestibular schwannomas. The growth or removal of these tumors results in deafness from injury to the cochlear nerves. Also candidates for the ABI are children with hypoplasia of the cochlea or cochlear nerve, and adults and children with scarring of the cochlea following trauma, otosclerosis, or meningitis (Colletti et al., 2009a; Noij et al., 2015; Puram et al., 2016). Altogether, over 1500 patients have been implanted since 1979, when the first patient, a woman with NF2, received a device with two ball electrodes placed on the CN (House and Hitselberger, 2001).

Only a small group of ABI users achieve the ultimate goal of an auditory prosthesis, open set sentence comprehension, which is the ability to understand speech without visual clues. The majority of ABI users only obtain general sound awareness that does aid in lip reading (Schwartz et al., 2008; Colletti et al., 2009b; Vincent, 2012). In contrast, most CI users attain much better performance and achieve some degree of open set speech comprehension. Psycho-physical experiments show that ABI and CI users perform comparably in detecting temporal cues (i.e. amplitude modulated signals), but ABI users perform much poorer at discriminating between frequencies (Shannon and Otto, 1990) and can have trouble ranking the pitches of their electrodes (Otto et al., 2002). Although contemporary ABI devices consist of an array of 12–21 electrodes placed on the surface of the CN, accurate frequency encoding may not be provided because of broad, overlapping regions of excitation of subsurface neurons. In addition, multiple subtypes of neurons in the CN are likely to be stimulated by the ABI in a mixture of tonotopic and non-tonotopic patterns. Such simultaneous activation likely contributes to minimal speech comprehension in ABI users. Other factors limiting ABI performance could be damage to the CN caused by a tumor and/or its removal (Colletti and Shannon, 2005) and non-optimal placement of the ABI electrode array (Barber et al., 2017).

The surface stimulation of the CN used in the human ABI has not previously been studied in detail in animal models. One study using two surface electrodes (McCreery et al., 2010) showed that responses are broader than those evoked by microstimulation within the CN using penetrating electrodes (McCreery et al., 2010; Shivdasani et al., 2008; Takahashi et al., 2005). Penetrating electrodes were used in a few human subjects but have been discontinued because of side effects (Otto et al., 2008). Surface electrodes almost certainly evoke responses in multiple types of CN neurons. One type that is tonotopically organized in the dorsal cochlear nucleus (DCN) is the pyramidal/fusiform neuron; these principal neurons are

arranged from low to high characteristic frequency (CF) along the long axis of the DCN (Kaltenbach and Lazor, 1991; Oertel and Young, 2004; Ryan et al., 1988; Spirou et al., 1993). Thus, ventrolateral electrodes are likely to activate mostly low-CF principal neurons and dorsomedial electrodes mostly high-CF principal neurons. However, this may not be the case for DCN interneurons, for fibers, and for the more remotely located neurons of the ventral subdivision (VCN). The present study used a strategy to tease out tonotopic patterns by subtracting activity that is non-tonotopic because it was “common”, or elicited in response to most or all electrode pairs. A second strategy was to subtract the late phase of response, because the early phase of response is likely to be from principal cells that have large diameter, fast-conducting axons (Adams, 1979; Baizer et al., 2012; Schofield and Coomes, 2005).

A further strategy to improve performance of surface stimulation was to use a flexible electrode array (Guex et al., 2015). This type of array can conform to the small radius of curvature of the rat DCN (radius = 1.65 mm) without a change in electrode impedance. The microelectrodes within the array are embedded in a few micron-thick plastic film. Microfabrication using thin-film micromachining techniques and polymers allows for the miniaturization of the ABI electrode array so that it can fit the small DCN in animal models without compromising electrode density. Indeed, the human clinical ABI made by Cochlear Corp. hosts 21 electrodes on an array that is 3 mm by 8 mm, while that used here carries 20 microelectrodes on an array that is 1.1 mm by 1.9 mm and fits the exposed DCN in the rat. Furthermore, its small electrodes (100 μm in diameter) are also smaller compared to the 550–700 μm diameter electrodes in the human ABI (Vincent, 2012). Finally, we also compared bipolar vs. monopolar stimulation along the CN’s tonotopic axis. These tests are motivated by the idea that bipolar stimulation produces a more focused excitation volume of the neural tissue, as observed in the cochlea (Middlebrooks and Snyder, 2007; Snyder et al., 2008; Zhu et al., 2012) and in deep brain stimulation (Kuncel and Grill, 2004; O’Suilleabhain et al., 2003). Although bipolar stimulation is not always associated with functional benefits in these studies, it can potentially reduce the lateral spread of stimulation current (Bonham and Litvak, 2008) and might thus reduce side effects.

2. Methods

2.1. Fabrication of the stimulation array

Electrode arrays were fabricated using standard micro-fabrication processes (Guex et al., 2015). A sacrificial layer of Ti (10 nm)/Al(100 μm) was first deposited by evaporation on a silicon wafer. A first layer of 4 μm thick polyimide (PI2611, HD Microsystems GmbH, Germany) was then spin-coated and cured (soft bake, 5 min at 120 °C followed by hard bake, 2 h at 300 °C in a N₂ oven). Next, the interconnects layer (Ti/Pt/Ti, 75/350/75 nm) was sputtered after O₂ plasma surface activation and patterned by photolithography and IBE (ion beam etching). A second layer of PI (4 μm thick) was subsequently spin-coated and cured, then patterned by photolithography and RIE (reactive ion etching), defining both the electrode active sites and the array’s external shape. The electrode arrays were released from the wafer by anodic dissolution of the Al layer (1 V bias, in saturated NaCl solution). Each array contains 20 electrodes patterned in a hexagonal configuration, with 4 rows, each of 5

electrodes (Fig. 1C). The total surface of the array is 1.1 mm by 1.9 mm, which was tailored to be the size of the exposed DCN in rat after cerebellar aspiration as observed in this study. The 100 μ m diameter electrodes, patterned on fingerlike stripes of PI, conform to the surface of the DCN (Fig. 1A–C). The array interconnects are routed to the side at caudal aspect of the DCN and are interfaced to a rigid cable and connector.

2.2. PEDOT coating of the electrodes

PEDOT: PSS was electropolymerized from a solution of EDOT (0.1% w/v) and PSS (0.2% w/v) (both purchased from Sigma Aldrich), as described (Guex et al., 2015). Electropolymerization was performed galvanostatically with a deposition charge of 75 mC/cm². The impedance modulus at 1 kHz of the coated microelectrodes was decreased by more than one order of magnitude compared to uncoated microelectrodes.

2.3. Surgery

All experimental procedures were performed in accordance with the National Institute of Health guidelines for the care and use of laboratory animals as well as approved animal care and use protocols at the Massachusetts Eye & Ear, Boston, MA. *In vivo* tests were performed on male Sprague-Dawley rats (350–500 g) within a sound-attenuating chamber. Animals were anesthetized with Ketamine (100 mg/kg) and Xylazine (20 mg/kg) for the duration of the experiment. The head was fixed, a left occipital craniotomy was performed, the dura mater was opened, and a small portion of the ipsilateral cerebellum was aspirated to expose the left DCN (Fig. 1A). A second craniotomy was performed over the right temporal-parietal suture, to access the dorsal surface of the right IC.

2.4. Placement of the stimulation array

The array was placed on the exposed surface of the left DCN (Fig. 1A). eABRs (Fig. 2A) were recorded differentially using two subcutaneous stainless steel needle electrodes, one placed on the vertex, the other behind the ipsilateral pinna, with a ground electrode placed on the back of the animal. Recordings were averaged over 512 stimulus presentations and filtered with an analog bandpass filter (30 Hz–3 kHz) amplified by 60 dB before A/D conversion with a sampling frequency of 25 kHz (Ithaco Model 1201, DL Instruments, Ithaca NY). Post-experiment histology confirmed accurate placement of the ABI electrode on DCN (Fig. 1B) by inserting pins (0.1 mm diameter Minutien) at the corners of the array and subsequent recovery of the pin-holes in Nissl-stained frozen sections.

2.5. Recordings of evoked multi-unit activity in the IC

A single-shank, 16-channel penetrating recording probe (A1×16–5 mm –150–177, Neuronexus, Ann Arbor, MI) was inserted into the right IC, the side contralateral to the stimulated DCN (Fig. 1D). Multi-unit activity was measured simultaneously from the 16 recording sites, first in response to acoustic stimulation (left ear tone pips, 20 ms duration; 1–46.5 kHz with 2 steps per octave; 0–80 dB SPL). Measured signals were sampled at 25 kHz and the average signal of all sites was used as a global reference and subtracted before further analysis. Responses were displayed as IC response maps (Fig. 3) in response to each of the acoustic frequencies. Stimulation with high acoustic frequencies induced activity in

the low-numbered recording sites (located ventromedially in the IC); as frequency was decreased, the activity progressively shifted to higher-numbered sites (more dorsolaterally; Figs. 1D and 3A) and a monotonic progression of response was evidence of the probe's location in the central nucleus of the IC. The logarithmic fit was used to obtain the characteristic frequency (CF) associated with each recording site (Fig. 3B, curves). Only the sites for which acoustically-evoked activity was recorded were considered for analysis of the electrically-generated data.

2.6. Electrical stimulation protocol

Electrical stimulation was induced with custom-made isolated current sources. Stimuli were current-controlled but expressed in voltage levels due to the inadvertent presence of a 2 k Ω resistor in parallel with the output during the experiments. Thus, although the exact stimulation current levels are unknown, they are estimated from combined electrode/tissue impedances. At the end of the experiment, we measured the electrode-tissue impedance systematically across the array with a PalmSens3 impedance analyzer and found average values of 395 k Ω at 1 kHz and 74 k Ω at 10 kHz ($n = 17$ electrodes). In saline solution, the typical electrode impedance was measured to be 2.66 k Ω at 1 kHz (Guex et al., 2015). Thus, a stimulation at 4 V (the maximal used) corresponds to a maximum current level of 0.01 mA flowing into the DCN.

Trains of charge-balanced biphasic symmetric pulses at 0.2 ms/phase were delivered to the CN at 28 pulses/s and alternating in polarity. Minimal differences were observed between the two different polarities so the average of the two polarities was used in the analysis presented here. Stimulation level ranged from 0 to 4 V in steps of 0.5 V. Baseline activity was recorded during the 500 ms off period after each 500 ms pulse train. Bipolar stimulation was applied between two electrode sites of the array, whereas monopolar stimulation was applied between one electrode in the array and a reference electrode (stainless steel needle placed in the neck muscles of the animal). Due to the hexagonal structure of the electrode sites on the array, three orientations were characterized, and defined by their angle with respect to the lateromedial axis (-60° , 0° , 60°). Two inter-electrode distances were tested, 400 μm and 800 μm . Monopolar and bipolar stimulation runs were performed in a pseudo-random order.

2.7. Multi-unit recordings processing and analysis

Processing of the data was performed with Matlab software (The Mathworks Inc., Natick, MA). The first 1 ms following each stimulus pulse onset was discarded to eliminate the stimulation artifact. A forward and reverse bandpass Butterworth filter of order 5 was applied, with cut-off frequencies of 500 Hz and 3 kHz. Spikes were detected by non-negative crossings of a threshold (Fig. 2C) set at three times the standard deviation of spontaneous activity. The evoked spike rate was determined after subtraction of the baseline spike rate. Averages of 30 repetitions of 500 ms pulse trains were obtained for each condition.

Spike rates were averaged across trial duration and stimulus repetitions. Post-stimulus time histograms (PSTHs) were obtained for each condition with a resolution of 0.1 ms.

Significance of activation for each recording site and stimulus intensity combination was determined by a student's t-test between spike rate during the stimulus on and stimulus off periods. Spike rate of non-significant conditions ($p < 0.001$) were set to zero to decrease the noise of the calculations.

The best threshold for each IC response map was determined as the lowest electrical stimulation level generating significant activity on at least one of the recording sites. For each level l above best threshold, the centroid of activity was calculated with the following formula (k : IC recording sites; MAP : 2-dimensional IC response map):

$$C(l) = \frac{\sum_k k \cdot MAP(k, l)}{\sum_k MAP(k, l)}$$

The width of the IC response map was defined for each level above best threshold as the double of the standard deviation of the centroid (Escabi et al., 2007), with IC recording site instead of octave frequency):

$$W(l) = 2 \cdot \sqrt{\frac{\sum_k (k - C(l))^2 \cdot MAP(k, l)}{\sum_k MAP(k, l)}}$$

The centroid for electrical stimulation was defined at a level of 0.5 mA above the best threshold ($C_e(T + 0.5 \text{ mA})$) for each IC response map. The centroid for acoustic stimulation was defined at 10 dB SPL above threshold ($C_a(T + 10 \text{ dB SPL})$) for each acoustic frequency. Rate-level curves were obtained using the average spike rates at the centroid $\pm 1/2$ width. Spike counts were obtained at each level by adding the evoked spikes over all IC recording sites. Rate-level curves and spike counts were normalized for each rat to the maximum of the rate level curves, respectively the maximum spike count across all stimulation conditions.

The common activity was calculated by averaging IC response maps with significant activity evoked by all electrode pairs across the DCN. This average map was calculated separately for the different inter-electrode distances. The early vs. late phase of response were distinguished qualitatively by PST histograms.

2.8. Statistical analyses

Results are expressed as mean $\pm$ standard error of the mean. Statistical analyses were performed on the data and significance was indicated by a p-value lower than 0.05. Pearson's correlation, multiple regression analysis, analysis of covariance (ANCOVA) and ANOVA were used, depending on the data.

3. Results

3.1. Responses to DCN electric stimulation

With the array placed on the surface of the DCN, all electrodes were observed to be in close contact with the neural tissue (Fig. 1A). Electrical stimulation using pairs of electrodes

evoked eABRs (Fig. 2A). The eABR waveform was variable, even for different electrode pairs within the same animal, and it consisted of 2–4 positive-going and negative-going peaks in succession in the 5 ms after the electrical pulse (Fig. 2A). The eABRs showed little change in threshold or suprathreshold amplitude with change in electrode location along the lateromedial (long) dimension of the array (as shown by matrices for one rat in Fig. 2B, and by graphs for all data in red in Fig. 2E). In contrast, along the rostrocaudal dimension, thresholds decreased and suprathreshold amplitudes increased caudally (Fig. 2F).

Electrical stimulation also evoked multiunit neural activity in the IC (Fig. 2C), with rates significantly greater than spontaneous activity in the majority of runs (372 out of 398, 93.5% across all animals). Like the eABRs, there was little change in multi-unit activity evoked by change in electrode location along the lateromedial dimension (Fig. 2D and E). In contrast, rostrocaudally, thresholds increased somewhat and normalized spike rate decreased caudally (Fig. 2F and Table 1), which is a pattern opposite to that for the eABR. Electrode pairs with the highest IC thresholds or no responses were almost always located on the rostral edge, which is shown by the darker colors toward to the tops of the matrices in Fig. 2D. Post-experiment histology suggests that these rostral-edge electrodes were at or just beyond the edge of the DCN (Fig. 1B).

3.2. Tonotopic patterns in raw responses

3.2.1. Patterns in responses evoked by sound—Each rat showed responses to individual sound frequencies over a narrow range of recording sites in the IC (Fig. 3A). Response maps were V-shaped. For each sound frequency, we calculated a centroid of activity that was usually at the tip of the V (Fig. 3A, color IC response map at top left). Plots of the centroids (Fig. 3B) showed orderly mappings that were similar across animals with an average of 1.92 recording sites per octave of frequency. The logarithmic fit to the data (Fig. 3B, curves), the tonotopic mapping, was used to assign a characteristic frequency (CF) to each recording site in the IC (see Fig. 4B, x axis), and these CFs were used to determine the mapping of response to electrical stimulation (Fig. 4C–E).

3.2.2. Patterns in responses evoked by electrical stimulation—Compared to the patterns in response to sound, those in response to electrical stimulation had more variability. The response areas were broader and some areas were “split” between two groups of IC recording sites (Fig. 4A, Rat #4). The centroid of response to electrical stimulation (Fig. 4B) varied with stimulus pair position along both the lateromedial and rostrocaudal dimensions of the DCN (Fig. 4C and D). The axis that maximizes the change in CF, the tonotopic axis (Fig. 4E), is a combination of the lateromedial and rostrocaudal array dimensions, consistent with the spatial mapping of sound-evoked responses cats (Spirou et al., 1993) and mice (Muniak et al., 2013). The mappings across all stimulating electrodes, though, showed ranges (avg. 1.03 octaves, 17.23–35.3 kHz) smaller than for sound stimulation (5.54 octaves, 1–46.5 kHz). They could be horizontal (e.g., Rat 1 in Fig. 4C), which indicates no change in CF with change in electrode pair position lateromedially. Overall, response CF was positively correlated with the electrode pair position in only 4/7 rats lateromedially but in 6/7 rats rostrocaudally (Fig. 4C and D, Table 1).

3.3. Tonotopic patterns after subtraction of common activity and late phase of response

3.3.1. Common activity—Such weak mappings could result from the responses being dominated by non-tonotopic activity that is evoked by all electrode pairs. To investigate this idea, we subtracted the “common activity” from the overall response. The common activity was calculated as an average IC response map; across rats, these maps had an average CF of 22.4 ± 1.5 kHz. The average map for one rat is shown in Fig. 5A (center IC response map). In this rat, the effect of subtraction on the response areas to two stimulus pairs was to narrow them and to shift their centroids to somewhat higher CFs (Fig. 5A left, red numbers on response maps). For all stimulus pairs, matrices (Fig. 5A right) show CFs for the raw data and after subtraction. Before subtraction, the range of CFs was 1.39 octaves (12–31.6 kHz), whereas after, the range of CFs was 2.67 octaves (8.1 kHz–51.5 kHz). Combined data (Fig. 6A) also showed this effect: a larger range (1.91 octaves after subtraction vs. 0.83 octaves before). Thus, the plot of CF vs. distance along the lateromedial (Fig. 6B) and tonotopic axes (Fig. 6B inset) both had significantly greater slopes after subtraction (ANCOVA). The direction of the tonotopic mapping, or “CF shift orientation”, was 68° for the raw data and was 51° after average subtraction.

3.3.2. Early vs. late phase of response—Weak mappings from electric stimulation might also result from activation of less tonotopic neurons having long latency. In contrast, short latency is a characteristic of the fast-conducting axons of the tonotopic DCN principal cells. Using latency, the response was separated into early phase and late phase activity using PST histograms (e.g. Fig. 5B). The cutoff between early vs. late phase of response was quite variable across electrode pairs (both within a single test subject and between subjects); its value ranged between 4.75 and 9.75 ms. The IC response maps in Fig. 5B show that the early phase had narrower response areas than the late phase. Across stimulating electrodes, the early phase activated IC recording sites over a greater range of CFs. In the case of the rat shown in Fig. 5B, early-phase CFs had a range of 2.67 octaves (8.1 kHz–51.4 kHz), whereas for the late phase CFs had a range of only 1.47 octaves (14–36.1 kHz), as shown in the matrix plots. Data from all seven rats showed this effect, with an average range of 1.20 octaves for early vs. 0.89 octaves for late, so that the CF-distance plot for early spikes showed a greater slope (Fig. 6E) and was significantly different with lateromedial position although not with tonotopic position (ANCOVA). Perhaps these different effects result from the large change in angle of the CF shift orientation (4° for early phase vs. 76° for the late phase).

3.3.3. Combined subtractions—A combined subtraction of the common activity and the late phase of response generated the CF plot with the steepest slope (Fig. 7A). This range of CFs (1.96 octaves) was slightly larger than just the average subtraction (1.88 octaves), and clearly larger than for the early phase of response, (1.07 octaves), with both significantly different than the combined (ANCOVA). The average CF shift orientation after combined subtraction was 12° (Fig. 7A inset), close to the CF shift orientation obtained with early latency spikes (4°) but very different than raw data (68°) and average subtraction (51°).

3.4. Widths of response areas

In raw, unprocessed responses, widths of IC response maps (Fig. 4A) to electrical stimulation were variable and usually broad. They averaged 6.4 ± 0.3 recording sites (3.33 octaves). Widths were significantly correlated to lateromedial position only in rats 5 and 7, with a narrower width on the lateral part of the implant. Width had significant rostrocaudal dependence for 6/7 rats (all except Rat 4, Table 1), with narrower width on the rostral side of the implant. After subtraction of the common activity, response maps became on average significantly narrower (multiple linear regression, $p < 0.05$, Fig. 5A), and the width showed more dependence on lateromedial position of electrode pair (Fig. 6C). The width was also significantly narrower for early phase compared to the late phase of response (mult. lin. reg, $p < 0.05$, Fig. 5B), but both phases showed almost no dependence on lateromedial position (Fig. 6F). The width after combined subtraction was narrower than after each individual subtraction ($p < 0.05$ in both cases, Fig. 7B). As in the data after subtraction of common activity, the width was significantly correlated to the lateromedial position ($p < 0.05$ in both cases), with lower widths at lateral positions. Overall, combining both subtractions produced response maps with a maximal range of CFs, minimal widths, and resulted in a near-lateromedial orientation of the tonotopic axis.

3.5. Effects of stimulating electrode distance and pair orientation

So far, data presented have used bipolar stimulation with an inter-electrode spacing of 400 μm . The IC responses to larger inter-electrode distance (800 μm), with the combined subtraction, had fewer rats (4/7 at 800 μm vs. 6/7 at 400 μm) with a significant correlation between CF and lateromedial position. The range of CFs was somewhat smaller (32.9 kHz vs. 39.6 kHz). The only effect of electrode pair orientation was that at 800 μm distance (Fig. 8B), the range of CFs was somewhat larger at a -60° orientation. However, effects of angle at either 400 or 800 μm spacing were not significant (ANCOVA). Larger inter-electrode distance also broadened the width of activation, for all orientations combined (ANOVA, $p < 0.05$). For a 400 μm distance, the width was on average 3.17 recording sites (1.65 octaves), whereas for 800 μm the width was on average 3.6 sites (1.88 octaves, Fig. 8D). Again, the only effect of electrode-pair orientation was for 800 μm distance, and again, the pairs with a -60° orientation were different (they generated a narrower width than the other orientations, ANOVA, $p < 0.05$). Variations of threshold and normalized spike count with distance and orientation showed no significant effect (Fig. 8C,–E; $p > 0.05$). In the raw data, however, there was a significantly larger spike rate with 800 μm distance (not illustrated)

3.6. Relative similarity of bipolar vs. monopolar stimulation

Response patterns to monopolar stimulation were generally similar to bipolar stimulation. These experiments used an additional 4 rats and 400 μm pair spacing for direct comparison. Fig. 9A shows in one rat the similar shapes of raw IC response areas for bipolar stimulation vs. monopolar stimulation (5 bipolar pairs vs. 10 corresponding monopolar electrodes). Similar spatial variations were observed both in raw data (Figs. 9B and 10A) and data with combined subtraction (Figs. 9C and 10B). There was no significant difference in the CF variation with the lateromedial position of stimulation between monopolar and bipolar data

(multiple linear regression, $p > 0.05$ both for the first order polarity effect and for the interaction polarity/position).

The main difference between bipolar and monopolar stimulation was that spike rates were significantly higher for the latter after combined subtraction (Fig. 10E; ANOVA, $p < 0.05$). This effect was also observed in the raw data but did not reach significance ($p > 0.05$). No significant differences were observed in best threshold or width of activation, both before and after subtractions (Fig. 10C and D; ANOVA, $p > 0.05$).

4. Discussion

Present results are the first detailed tests of surface stimulation in an animal model of the human ABI. The unprocessed responses had limited tonotopy and were usually wide. These results offer one explanation for the difficulties that ABI users have with frequency discrimination (Shannon and Otto, 1990), and they illustrate the challenges for presenting frequency-specific cues in this neuroprosthesis.

4.1. Microfabricated electrode arrays for the ABI

Thin film electrode technology allows for miniaturization and increasing the density of electrode sites (Allitt et al., 2016; Rodger et al., 2008). The thin-film array tested here, using the technology described previously (Guex et al., 2015), accommodates 20 microelectrodes within 2 mm^2 , an electrode density much higher than that of clinical ABIs. Its success was demonstrated by the fact that activity was evoked by nearly all ($>93\%$) of the electrodes. Given that the rat CN is about 1/16 the size of the human auditory brainstem (Rosahl and Rosahl, 2013), these results suggest that a clinical ABI incorporating a much larger number of electrodes could be effective in evoking responses in humans. However, such microelectrodes ($<1 \text{ mm}^2$) will need to be optimized against safe charge injection and long-term *in vivo* use (McCreery et al., 1990). The surface of neural tissues is usually curvilinear, so we used a strategy to increase the proximity of the electrodes by the use of thin and plastic materials (Baek et al., 2014; Kim et al., 2010; Minev et al., 2015). Furthermore, matching not only the contour and static mechanical response of the tissue but also its dynamics (tissues constantly move due to respiration and cardiac pulsation) opens the path to implants that are better biointegrated in the long term (Minev et al., 2015). The array used here is conformable, providing close contact of the ABI microelectrodes with the curved surface of the DCN (Fig. 1A, Guex et al., 2015). This characteristic may produce the observed low and uniform pattern of thresholds across the DCN's long lateromedial surface (Fig. 2E), although this conclusion must be tempered somewhat since: 1) the responses were not explicitly compared to those of a rigid array, and 2) stimulation voltage levels were reported and the exact stimulation current levels are not known. In some human subjects, there is an uneven pattern of response in which the electrodes at the center of the array have lower thresholds than those at more peripheral locations (Barber et al., 2017). This pattern may result from the human ABI array being less conformable so that only the center electrodes are close to the neural tissue. Unfortunately, the higher currents necessary for auditory responses at the periphery of the array may be responsible for activation of other nearby structures in the brainstem. In fact, side effects are a common problem in adult ABI

users (Herrmann et al., 2015; McCreery et al., 1998; Ramsden et al., 2016; Wilkinson et al., 2016) and require that such electrodes be turned off during programming. Thus, conformable arrays for a next-generation human ABI may be an important step toward fewer side effects and thus more useable electrodes to provide tonotopy

4.2. Tonotopy from surface stimulation of the cochlear nucleus

A challenge for the ABI is to provide tonotopic patterns of response from surface stimulation. Unfortunately, even with a conformable array hosting small electrodes, the unprocessed responses to electric stimulation were not as narrow as those evoked by pure-tone acoustic stimulation (Fig. 3A) or by microstimulation within the CN (Mauger et al., 2010; McCreery, 2008; Shivdasani et al., 2008). Processed responses did have larger ranges of CFs and narrower widths. The first processing used, subtraction of common activity, ignores and perhaps even distorts temporal patterns of response. For that reason the second processing, subtraction of the late phase of response, has its own advantages to reveal the contributions of tonotopically organized neurons such as the DCN principal cells. These cells are almost certainly activated by the array placements used here, because they are located in layer II, just a few hundred micrometers below the surface (Willard and Ryugo, 1983). Their tonotopic patterns are likely to be projected centrally into the IC. Future stimulus paradigms might be designed to increase the activity in the pathways providing tonotopic cues.

There are important caveats in extending our animal results to the human ABI. The animal results were obtained in preparations having functional auditory nerve fibers, whereas most ABI users have none. Conceivably, the nerve fibers could have responded to the electric stimulation and carried the response to distant parts of the CN, blurring the tonotopy. In addition, long-term hearing impairment, such as that in NF2, causes plastic changes in the CN (Ryugo, 2015; Salvi et al., 2000), and these effects were not present in our normal-hearing animals. Finally, there may be functional differences between animals and humans, since the DCN is: 1) layered in animals but appears to be unlayered in humans, and 2) contains in animals a prominent type of neuron called granule cells that may be lacking in humans (Baizer et al., 2014).

4.3. ABI stimulation of DCN vs. VCN

In our study, we placed the electrode array on a clearly visible DCN; thus, components of the IC neural response almost certainly result from DCN principal cells. One idea is that other, non-tonotopic components, as well as the eABR, result from stimulation of the VCN, which is adjacent and shares a common boundary with the DCN (Fig. 1B). In support of this idea, the ABR, which is the response to sound stimuli, is generated by VCN bushy cells and their pathways (Melcher and Kiang, 1996). If the eABR is also generated by the VCN, it would explain its distinct spatial variation compared to the IC neural responses found in the present study (Fig. 2F). The identity of the generators of the eABR is an important clinical issue, because the eABR is the functional marker used to adjust the position of the ABI electrode array in humans (the surface of the CN is not visible to the surgeon). Bushy cells are thought to be part of a neural pathway mediating binaural localization of sound sources, having excellent phase locking and projections into centers in the superior olivary complex

where neurons are sensitive to interaural level and interaural time differences (Smith et al., 1993). However, their contributions to speech perception are unknown. Busy cells might be responsible for the late-phase IC neural responses (Fig. 5B) because their ascending pathway includes a synapse before arriving at the IC (Cant and Benson, 2003). The late-phase responses could also be mediated by VCN “T” stellate cells that project to IC via thin, slow-conducting axons. These possibilities are consistent with microstimulation within the VCN that results in a late phase of activity (Shivdasani et al., 2008), and with lesion studies in mice showing the early phase is eliminated yet the late phase remains after section of the DCN’s output pathway (McInturff et al., 2018). Additional contributors to the late phase could be less tonotopically organized pathways beginning within the DCN (perhaps involving parallel fibers or the auditory nerve fibers that remain in our preparation)

4.4. Electrode spacing, angle, and monopolar vs. bipolar stimulation

The dense packing of electrodes on the array allowed us to compare closely spaced vs. more distantly spaced electrodes in a bipolar pair. The distantly spaced pairs evoked a less tonotopic pattern of responses and also broadened the width of activation. These results suggest that if bipolar stimulation were used in the clinical ABI, reducing the inter-electrodes distance might improve tonotopic representations. For the rat CN, even the distantly spaced pairs (800 μ m) are still closer than for the spacing in the available clinical ABI electrode array (about 1 mm), suggesting that the tonotopy provided by the clinical device is particularly low.

The DCN principal neurons have axons and dendrites travelling perpendicular to the ventrolateral-to-dorsomedial tonotopic axis, and thus may be activated differently with current pulses applied with various orientations. As shown in the data obtained with the larger inter-electrode distance (800 μ m), the orientation of the pairs may influence the shaping of the current field and volume of neural tissue that is activated in CN (Bonham and Litvak, 2008), resulting in varying tonotopic organization of responses. Electrode pair orientations with negative angles (close to orthogonal to the tonotopic axis after combined subtraction) had two differences from other orientations: they resulted in activation of a larger range of recording sites along the IC’s tonotopic axis, and the widths of the activated areas were narrower. Perhaps this results from the long axis of the electric field being perpendicular to the DCN’s tonotopic axis. If bipolar stimulation were used in the clinical ABI, pair orientation should be chosen to be perpendicular to the tonotopic axis.

Our data indicate that bipolar electrode configurations alone do not usually result in narrower receptive fields compared to the monopolar condition (Figs. 9 and 10D). Such a result might suggest that the volume of tissue activated by the electric field is roughly similar for the two conditions. One possibility is that for bipolar configuration, much of the current path is directly from one electrode to the other through the superficially-most tissue of layer I, and thus avoiding the principal cells. This idea is consistent with our finding that spike rates in response to monopolar stimulation were on average higher than to bipolar stimulation. Another possibility is that in biphasic stimulation both electrodes of a bipolar pair act as cathodes/anodes. Contrary to studies comparing monopolar and bipolar stimulation in the cochlea (Allitt et al., 2016; Bonham and Litvak, 2008; Middlebrooks and

Snyder, 2007; Snyder et al., 2008; Zhu et al., 2012), the threshold obtained with bipolar stimulation is not higher than with monopolar stimulation. In the human ABI, bipolar stimulation is used intra-operatively to assist the positioning of the ABI array, but everyday use employs monopolar stimulation to minimize power consumption (Herrmann et al., 2015).

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Fig. 1. Stimulating array and recording setup. A) Photograph of the miniaturized, conformable stimulating electrode array in position on the left DCN in a rat. B) Overlaid electrode array schematics from 5 rats, pictured on a color CN reconstruction (from Verma et al., 2014). Schematics were positioned according to post-experiment histology (see Methods). The colored arrow indicates the orientation of the lateromedial axis along the roughly lateroventral-to-mediadorsal dimension of the DCN. C) Photograph of electrode array before positioning, showing the electrode pair spacings and orientations that were tested. D) Schematic showing the recording probe in the IC contralateral to the stimulated side. In the IC, the tonotopic axis runs from low characteristic frequencies (CFs) dorsolateral (dark colors) to high CFs ventromedial (light colors). Left-ear sounds presented at the beginning of each experiment confirmed placement along the tonotopic axis of the IC and determined the CF of the individual recording sites. Subsequently, electrical stimulation of electrode pairs in the DCN evoked IC responses as well as eABRs (recorded by cutaneous electrodes that are not shown). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 2.

Waveforms and spatial variations. A) Example electrically-generated auditory brainstem response (eABR) showing 4 waveform peaks. The stimulation pulse started at time 1 ms and in this trace the stimulation artifact was very small. B) eABR thresholds (left) and suprathreshold amplitudes (right) plotted as color-coded matrices, replicating the view of the array on the DCN (Fig. 1A–C). The matrix x and y axes are the lateromedial and rostrocaudal dimensions, respectively (see arrows). Data are for individual Rat #5. Suprathreshold amplitudes are eABR normalized RMS energy (of the first 10 ms of the

signals 1 V above threshold). NF represents a non-functional electrode on this array. C) Example multi-unit waveform from one IC recording site after filtering. The trigger level for spike detection is shown by the green horizontal line. The first 1 ms of the trace, which contains the stimulation artifact, was deleted (spike latencies were longer than 1 ms). D) Color-coded matrices as in B but here for IC spike count threshold and a suprathreshold measure (normalized IC spike count at 1 V above threshold). Thin dashed lines represent electrode pairs producing no significant response. E) Comparison of lateromedial spatial variations of eABR and IC thresholds (left) and suprathreshold spike count (right), for the individual rat (panels B,D) and for the average of all rats. “Coordinate” on the x axes refers to distance from center of the array (0) to the midpoint of the bipolar electrode pair (see matrices above) with negative values lying laterally and positive values medially (arrow). F) Rostrocaudal spatial variations of eABR and IC threshold (left) and suprathreshold spike count (right). The x-axis here (rostrocaudal dimension) was the y-axis of the matrices (panels B,D). Negative coordinate values lie rostrally and positive lie caudally (arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 3.

Acoustic IC response maps in 7 individual rats show restricted response areas with clear tonotopic shifts. A) Response patterns in the IC following acoustic stimulation at 1, 4, 8, 16 and 32 kHz shown for each of the seven rats. These response maps have colors that correspond to firing rates (color scales) as a function of stimulation level and plotted for each of the 16 IC recording sites. A shift in activity along the recording probe is observed, from low site numbers at high frequency to high site numbers at low frequency. In top, left panel, red arrow indicates centroid of activity. B) The mapping of acoustic frequency vs.

centroid is the tonotopic mapping. Data points show the IC centroids corresponding to each acoustic frequency tested. Lines show logarithmic fit to the data, which determines the characteristic frequency (CF) for each IC recording site. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 4.

Electrical IC response maps in 7 rats show variable regions with less tonotopic shift than for acoustic responses. A) Top: Five bipolar pairs of stimulating electrodes (red, spacing 400 μm) chosen because they span the lateromedial and rostrocaudal distances along the DCN. Bottom: Response maps (as in Fig. 3) to electrical stimulation applied by the five bipolar pairs in seven rats. B) Example response map indicating the features that were extracted for analysis (see Methods), with centroid and width computed at 1 V above best threshold. The scale at the bottom shows the tonotopic mapping of IC recording sites to CF, which was

determined for each animal using acoustic stimulation (see Fig. 3 and Methods). C, D) CF as a function of DCN coordinate for all bipolar pairs (averages of all orientations at the particular distance point) across the seven rats. Response CF is positively correlated with the lateromedial electrode pair position in rats 2, 3, 4 and 5 and with rostrocaudal position in rats 1, 2, 3, 4, 5 and 6 (see Table 1). E) CF as a function of distance along the tonotopic axis, which was determined by performing a multiple linear regression of CF vs. distance along the lateromedial and rostrocaudal axes. Each point corresponds to a bipolar pair. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 5.

Subtraction of common activity and long-latency responses in an individual rat (Rat #5). A) Center: Average IC response map to all bipolar electrode pairs in this animal. Left: two response maps using two bipolar pairs of stimulating electrodes (red, above) that differ in lateromedial position. The raw responses and the responses after subtraction are shown. Subtraction results in narrowing of the response areas and small shifts of CF (red numerals). Matrices to the right: CFs for all electrode pairs using color-coded segments (see scales) between the two electrodes of each pair. The pattern illustrates the CFs across the DCN's lateromedial (x axis) and rostrocaudal (y axis) dimensions before (above) and after (below) the subtraction of common activity using the average response map. Thin dashed lines represent electrode pairs producing insufficient response to estimate the CF. NF represents a non-functional electrode on this array. B) Center PST: Responses separated into early and late phases according to a latency cutoff. Left: Response maps showing early phase is narrower than late phase for the two electrode pairs at top. Matrices on the right show the CF pattern for all pairs. Thin dashed lines represent electrode pairs producing insufficient response to estimate the CF. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 6.

Subtraction of common activity results in larger shifts of CFs and widths of activation; early phase responses have larger shifts and narrower widths. A) Color-coded matrices of CFs (left) before (top) and after (bottom) subtraction of average activity. The CF shift orientations (dotted lines) are shown. B, C) Plots of CF and width before and after average subtraction as a function of lateromedial position or tonotopic axis position (inset). After average map subtraction, the response CF of individual maps is positively correlated with the electrode pair position along the lateromedial axis in 5/7 rats (vs. 4/7 before subtraction). Multiple regression analysis demonstrate significant interactions between before vs. after average subtraction and the lateromedial coordinate ($p < 0.05$). Shaded areas are calculated with the regression of the top and bottom of the standard errors. D, E) Similar data after separation of early and late phases of response. Multiple regression analysis demonstrates significant interactions between early vs. late and the lateromedial coordinate ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 7. Combined subtraction of common activity and late phase of response shows the largest shifts of CFs. Plots of CF (A) and width (B) after separate and combined subtractions as a function of lateromedial position or tonotopic axis position (inset plots).

Fig. 8.

Response measures for inter-electrode distances of 400 vs. 800 μm . A) Plot of CF dependence for pairs with the three orientations (-60° , 0° and 60° , as shown in Fig. 1C). B) Plot of CF dependence for 400 μm and 800 μm electrode distance (also shown in Fig. 1C). C–E) Effect of distance and angle of the stimulation pair on measured best threshold (D), width of activation (E) and normalized spike count (F). Significant differences ($p\text{-value} < 0.05$) are indicated with a star.

Fig. 9. Similarities of responses to monopolar and bipolar stimulation in an individual rat (Rat #11). A) IC response maps (raw data) to bipolar electrical stimulation with the left column of plots showing five different bipolar pairs selected along the lateromedial axis and the middle and right columns of plots showing the response to monopolar stimulating using the corresponding individual electrodes. Red arrows and numerals indicate centroids of activity. B) CF matrices (raw data) for bipolar stimulation (top) or monopolar stimulation (bottom). C) CF matrices (after combined subtraction) for bipolar stimulation (top) or monopolar stimulation (bottom). Thin dashed lines represent electrode pairs producing insufficient response to estimate the CF. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 10.

Similarities of responses to monopolar and bipolar stimulation in all rats. CF dependence of (A) raw responses and (B) after combined subtraction, for monopolar and bipolar stimulation. Bar graphs of best threshold (C), width of activation (D) and normalized spike rate (E) for monopolar and bipolar stimulations, before (raw data) and after combined subtraction.

Table 1

P-values of the multiple linear regression tests.

P-values of the multiple linear regression of the best threshold (T), normalized spike count (NSC), CF, and width of activation (W) for lateromedial (lm) and rostrocaudal (rc) position of the stimulation pair. Values are also given for the interaction factor (int).

	T-lm Fig. 2E	T-rc Fig. 2F	T-int	NSC-lm Fig. 2E	NSC-rc Fig. 2F	NSC-int	CF-lm Fig. 4C	CF-rc Fig. 4D	CF-int	W-lm Fig. 6A	W-rc	W-int
1	0.505	0.382	0.015	1.4 ^{e-5}	0.034	0.030	0.218	3.9 ^{e-3}	1 ^{e-4}	0.102	7.7 ^{e-3}	5.7 ^{e-3}
2	0.543	0.174	0.550	0.010	0.024	5.8 ^{e-3}	3.1 ^{e-3}	2.1 ^{e-3}	0.092	0.060	5.6 ^{e-3}	0.079
3	0.078	0.316	0.202	0.021	1.6 ^{e-4}	0.833	0.029	9 ^{e-8}	0.752	0.281	1.2 ^{e-7}	0.060
4	0.220	0.021	0.478	1.4 ^{e-4}	3.7 ^{e-5}	0.065	1.7 ^{e-5}	0.023	0.073	0.308	0.661	7.5 ^{e-3}
5	3.8 ^{e-3}	3.5 ^{e-6}	0.750	1.4 ^{e-9}	2.2 ^{e-13}	2 ^{e-3}	8.9 ^{e-5}	1.4 ^{e-8}	0.102	5.5 ^{e-4}	1.2 ^{e-8}	0.368
6	2.3 ^{e-3}	2.6 ^{e-3}	0.920	5.2 ^{e-9}	5.6 ^{e-22}	1.9 ^{e-3}	0.079	8.8 ^{e-8}	0.647	0.461	2.3 ^{e-6}	0.809
7	0.536	4.1 ^{e-3}	0.027	4.1 ^{e-8}	1.4 ^{e-7}	1.2 ^{e-4}	0.144	0.155	0.564	3.9 ^{e-4}	0.030	0.382