The Summating Potential Is a Reliable Marker of Electrode Position in Electrocochleography: Cochlear Implant as a Theragnostic Probe

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Objective: For the increasing number of cochlear implantations in subjects with residual hearing, hearing preservation, and thus the prevention of implantation trauma, is crucial. A method for monitoring the intracochlear position of a cochlear implant (CI) and early indication of imminent cochlear trauma would help to assist the surgeon to achieve this goal. The aim of this study was to evaluate the reliability of the different electric components recorded by an intracochlear electrocochleography (ECochG) as markers for the cochleotopic position of a CI. The measurements were made directly from the CI, combining intrasurgical diagnostics with the therapeutical use of the CI, thus, turning the CI into a “theragnostic probe.”

Design: Intracochlear ECochGs were measured in 10 Dunkin Hartley guinea pigs of either sex, with normal auditory brainstem response thresholds. All subjects were fully implanted (4 to 5 mm) with a custom six contact CI. The ECochG was recorded simultaneously from all six contacts with monopolar configuration (retroauricular reference electrode). The gross ECochG signal was filtered off-line to separate three of its main components: compound action potential, cochlear microphonic, and summating potential (SP). Additionally, five cochleae were harvested and histologically processed to access the spatial position of the CI contacts. Both ECochG data and histological reconstructions of the electrode position were fitted with the Greenwood function to verify the reliability of the deduced cochleotopic position of the CI.

Results: SPs could be used as suitable markers for the frequency position of the recording electrode with an accuracy of ±1/4 octave in the functioning cochlea, verified by histology. Cochlear microphonics showed a dependency on electrode position but were less reliable as positional markers. Compound action potentials were not suitable for CI position information but were sensitive to “cochlear health” (e.g., insertion trauma).

Conclusions: SPs directly recorded from the contacts of a CI during surgery can be used to access the intracochlear frequency position of the CI. Using SP monitoring, implantation may be stopped before penetrating function cochlear regions. If the technique was similarly effective in humans, it could prevent implantation trauma and increase hearing preservation during CI surgery. Diagnostic hardware and software for recording biological signals with a CI without filter limitations might be a valuable add-on to the portfolios of CI manufacturers.

Key words: Cochlear implant, Cochlear microphonic, Compound action potential, ECochG, Guinea pig, Summating potential, Theragnostic probe

INTRODUCTION

Recently, indication criteria for cochlear implantation were expanded to include subjects with significant residual hearing (Miranda et al. 2014). The main issues in these subjects include preserving residual hearing and minimizing insertion trauma caused by the cochlear implant (CI). To ensure the beneficial interaction of acoustic and electric stimulation modes at the level of the auditory nerve (Tillein et al. 2015), implantation procedures based on the assessment of the individual cochlear morphology have been introduced (Rask-Andersen et al. 2012; Erixon & Rask-Andersen 2013; Avci et al. 2014; Würfel et al. 2014). To date, the intracochlear cochleotopic electrode position cannot reliably be determined during implantation. As a rule, surgeons have neither intraoperative real-time feedback on the basilar membrane function nor any procedures available that help to detect and prevent imminent cochlear damage. Traumata often become apparent only after auditory structures have already been irreversibly damaged (Dalbert et al. 2015b; Adunka et al. 2016). Thus, surgery still carries a risk of intraoperative trauma and penetration of functional cochlear regions with the CI. Methods for monitoring inner ear function as well as for determining the inner ear electrode position during cochlear implantation are required.

Recent approaches involve the recording of sound-induced cochlear responses measured from extracochlear sites, such as the round window. Others record the responses from inside the cochlea, using various kinds of electrodes, including the CI itself (Adunka et al. 2010; Choudhury et al. 2012; DeMason et al. 2012; Radellof et al. 2012; Calloway et al. 2014; Acharya et al. 2016). Because of the signal’s multiple generators, the interpretation of the cochlear response is intricate, and the determination of the electrode’s intracochlear position relative to the responding partitions of the organ of Corti in residually hearing conditions remains challenging.

To infer electrode position from intracochlear electrocochleography (ECochG), five main components require distinction: (1) an alternating current (a.c.) component, the cochlear microphonic potential (CM), and (2) the direct current (d.c.) component, the summating potential (SP), both considered to originate from hair cells (Whitfield & Ross 1965; Dallos 1973, 1986; Patuzzi et al. 1989; Zheng et al. 1997; Durrant et al. 1998; Yizhar et al. 2011; Forgues et al. 2014; Ramamoorthy et al. 2014), (3) a transient component at the beginning and at the end of the gross response, the compound action potential (CAP), which is generated by auditory nerve fibers (Eggermont 1976a; Möller & Jannetta 1981), (4) another a.c. component, the auditory nerve neurophonic potential (ANN), constituting the frequency after response of the auditory nerve at low frequencies generated by a subset of nerve fibers (Snyder & Schreiner 1984; Calloway et al. 2014; Forgues et al. 2014) and (5) a neural d.c. contribution (van Emst et al. 1995; Sellick et al. 2003; Forgues et al. 2014) termed the auditory nerve sustained potential (Forgues et al. 2014) that is dependent on the presence
of an intact cochlear nucleus (Sellick et al. 2003). In the present study, ANN responses were not separated from CMs.

Because of the place–frequency relation (i.e., cochleotopic organization) in primary auditory structures of the inner ear, all components of the ECochG carry some cochleotopic information, with the limitation that the CAP is generated in the internal auditory meatus and, therefore, carries very little information regarding the position of an intracochlear electrode (Eggermont 1976b; van Deelen & Smoorenburg 1986; Brown & Patuzzi 2010; Rattay et al. 2013). Current concepts assume that a close-by or even intracochlear position of recording electrodes provide higher positional sensitivity than extracochlear recordings. The challenge is the development of a reliable method to extract this information.

The present study establishes a method to distinguish the frequency-specific effects that depend on the intracochlear recording position from those responses that are not spatially restricted. We recorded the ECochG in response to tonal stimulation in parallel from 6 positions within the hearing guinea pig cochlea. For this purpose, we used a custom-made flexible CI array comparable to CIs used in clinical applications. The positional data deduced from the separated components of the ECochG was validated by the positional information gained from histological preparations of the implanted cochleae and by the guinea pig Greenwood function (Greenwood 1990). By this approach, an intracochlear electrophysiological “potential map” was determined. This map could in the future help to develop novel functional monitoring techniques to determine the intracochlear electrode position during CI surgery.

The possibility to record local ECochG signals directly from the CI contacts that are designed for stimulation purposes, turns the CI from a purely therapeutic device into a diagnostic tool or “theragnostic probe.” Future developments of hardware and software components that enable recordings directly from the CI for diagnostic purposes are, however, necessary.

MATERIALS AND METHODS

Animals

We used Dunkin–Hartley guinea pigs (n = 10) of either sex with presurgically normal auditory brainstem response (ABR) thresholds (see ABR measurements) for the experiments. The weight ranged between 345 and 615 g (mean: 440 g). All animals were handled and housed according to German (TierSchG, BGBl. I S. 1206, 1313) and European Union (Directive 2010/63/EU) guidelines for animal research and were legally approved by German state authorities and the institute’s animal welfare officer.

Animal Anesthesia and Monitoring

For initial anesthesia, the animals were injected intramuscularly with a mixture of 50 mg/kg ketamine (CP Pharma, Burgdorf, Germany) and 10 mg/kg xylazine (WDT, Garbsen, Germany) in solution. This initial dose additionally contained 0.1 mg/kg atropine sulfate (B. Braun Melsungen AG, Melsungen, Germany) to prevent mucous airway obstruction during the experiment. For maintaining adequate anesthesia, 20% to 30% of the initial ketamine–xylazine dose was injected in regular intervals throughout the surgical preparation. A tracheotomy was performed at the beginning of surgery for ventilation using a rodent ventilator (Ugo Basile, Gemonio, Italy). Tidal volume was adjusted to the animal’s body weight (Kleinman & Radford 1964). Respiratory rate was set between 50 and 60 per minute depending on end-tidal CO 2 concentration (kept between 3% and 4%).

After surgery, xylazine concentration was reduced to 5 mg/kg to prevent cardiorespiratory depression. Anesthesia level, appropriate ventilation and cardiovascular condition were continuously monitored by electrocardiography and capnometry. Additionally, the paw-pinch withdrawal reflex was tested at regular intervals to verify anesthesia level.

The animals' body temperature was continuously monitored by a rectal probe and maintained between 37°C and 38°C using a heating pad (Minco, Minneapolis, MN; and TC-1000 Temperature Controller, CWE Inc., Ardmore).

Surgery

Preparation and recordings from left and right ears were carried out sequentially in a soundproof chamber. The head of the anesthetized animal was secured in a customized rodent head holder that allowed adjustment along three axes. After local anesthesia (2% lidocaine), removal of the pinna, and dissection of the overlying soft tissue, the bulla tympani was exposed and opened with a hollow needle under stereomicroscopic visualization (Carl Zeiss OPMI pico, Carl Zeiss, Goettingen, Germany). The lateral bulla wall was removed using a micro-rongeur until the round window and the promontory were sufficiently exposed, without damaging the tympanic membrane or other middle ear structures. Using a diamond drilling burr (0.6 mm diameter), a low-speed cochleostomy was performed in the anterior–inferior region just below the rim of the round window. Particular care was applied not to damage the inner ear structures and not to induce any bleeding. A little leakage of perilymph was allowed to prevent the penetration of bone dust into the cochlea. A custom-made guinea pig CI (Fig. 1A; MedEl comp., Innsbruck, Austria; see below for further specifications) was gently introduced into the basal cochlear turn. Insertion was stopped when all six contacts had been inserted and the black square marker was located at the cochleostomy level (insertion depth =5 to 6 mm; Fig. 1A, B) or whenever a first resistance was felt. For better electrode stabilization, the silastic carrier of the implant was glued to the rim of the tympanic bulla (histoacryl; B. Braun Melsungen AG, Melsungen, Germany).

ABR Measurements

The guinea pigs’ auditory sensitivity was evaluated preoperatively and before and after cochleostomy by recording click-evoked ABRs. Three silver wire electrodes were subcutaneously inserted posterior to the tested ear (reference) to the vertex at the interorbicular line (active) and to the neck (ground). Condensation clicks of increasing intensity (0 to 80 dB SPL; 5 dB steps) and with a duration of 50 μs were presented using a dynamic speaker (DT48 Beyerdynamic, Heilbronn, Germany) placed approximately 1 cm in front of the outer ear canal. The signals were digitally generated by the AudiologLab data acquisition software (Otoconsult Comp., Frankfurt/M., Germany), running on a computer connected to a custom-built attenuator (PNS1; Otoconsult Comp.) and a 32-channel MIO card (NI-6259,
Intracochlear Measurements

The recorded signals were filtered (Butterworth filter 6th order, high-pass filter frequency 200 Hz, low-pass filter frequency 5 kHz) and amplified by 100 dB (100,000 times) using a filter and amplifier combination (F1 device; Otoconsult Comp.). One hundred recordings were averaged. Middle ear and cochlear function were considered normal for ABR thresholds below 40 dB SPL. All investigated animals satisfied this criterion before surgical preparation.

Intracochlear Measurements

Clicks and pure-tone burst stimuli were generated by the stimulation and acquisition software described above. The loudspeaker was placed in close proximity to the entrance of the external auditory canal using a conical adapter without displacing or obstructing the ear canal. Calibration of the sound level at the ear canal was performed using 1/4-inch microphone (type 4939; B & K, Nærum, Denmark) in combination with a preamplifier (type 2670; B & K) and a conditioning amplifier (type 2690, Nexus conditioning amplifier; B & K) connected to the National Instruments data acquisition board. The acoustic condensation clicks (50 μs) for intracochlear recordings were presented at increasing intensities (0 to 90 dB SPL with steps of 5 dB) with 50 repetitions each. Tone bursts for the ECochG ranged from 1 to 32 kHz (rise/fall time 5 ms, total duration 50 ms) and were presented in randomized order, with logarithmic increments of 4 steps per octave at intensities from 0 to 90 dB SPL. Between 4 and 32 kHz, we additionally sampled with 12 steps per octave. Averaged responses were determined from 10 stimulus presentations. Intracochlear signals were recorded using a six-channel guinea pig electrode (custom made to our requirements by Med-El, Innsbruck, Austria). The electrode had a contact spacing of 700 μm and a maximum diameter of 0.5 mm, which tapered to 0.3 mm at the tip (Fig. 1A). The insertion length was approximately 6 mm (Fig. 1B). Signals were acquired through the AudiologyLab software, using a custom electrode connector box with a Lynx-8 amplifier system (Neuralynx, Bozeman, MT). The signals were filtered (zero-phase digital filtering, IIR-filter: Butterworth filter, 6th order, 12 dB/octave) between 10 Hz and 9 kHz (upper hardware limit) and amplified 5000 times.

Histology

A subset of five CI-implanted cochleae was harvested for histological preparation. The silastic carrier of the implant was first secured to the rim of the tympanic bulla using tissue glue (Histoacryl, B. Braun Melsungen AG, Melsungen, Germany). The temporal bone was subsequently removed from the skull. The specimens were fixed in 10% formalin, dehydrated in ethanol using serial concentrations progressing from 70% to 99%, and immersed in degassed epoxy resin to achieve acrylic fixation. Vacuum was applied so that the epoxy mixture infiltrated the cochlea completely. After embedding, the specimens were X-rayed to assess the correct plane for sectioning. Excess portions of the temporal bone specimens within the epoxy blocks were trimmed to leave only the cochlea. After appropriate orientation, the resized blocks were re-embedded such that the cochlear central axis was oriented parallel to the plane of sectioning, which is perpendicular to the electrode array. The cochlear specimens were serially sectioned using a microgrinding technique (for a detailed description, see Stöver et al. 2005) with a “section” thickness of 200 μm. This technique allows the CI to remain in place and to be sectioned in situ. Each section was polished and stained with toluidine blue and photographed under a light microscope. We performed this procedure only in a subset of the animals since we typically reused the CIs.

For the analysis of the microgrinding data, we imported each stacked image sequence into the visualization software platform AMIRA 3D (Version 6.01, FEI, Hillsboro). The images were aligned using the automated alignment module of the software. The edge of the osseous spiral lamina, the electrode path in the cochlea, and the positions of the six electrode contacts were reconstructed from the images (Fig. 2A). We calculated the length of the outer edge of the osseous spiral lamina and the electrode insertion depth from the cochleostomy to the tip by vector addition of the respective data points. Thereby, we corrected for errors resulting from the microgrinding technique using the deviation of the reconstructed distance between the electrode contacts and the known center distance of 700 μm. We could not reconstruct the apical region of the cochlea exactly from the microgrinding images, but always got a good reconstruction of the basal hook region at the round window. Therefore, we took a published full cochlear reconstruction as
reference (Hofman et al. 2009) and calculated the percentage of missing length at the apex from the number of turns visible in the 2D projection of our reconstructions (Fig. 2B) and added this value to the reconstructed cochlear lengths. The hook region was excluded, and the frequency positions along the percentual length cochlea were calculated according to the Greenwood function (Greenwood 1990). From the relative length, a case to case calculation of the tonotopic positions of the CI contacts was performed. Thus, the theoretical intracochlear frequency position for the CI contacts could be inferred and compared to the electrophysiologically gained data.

Data Analysis

Recorded data were analyzed using custom-made Matlab routines (MathWorks, Natick, MA). We differentiated CAPs, CMs, and SPs within the recorded signals (Fig. 3A, B). The ANN was not considered separately and might have become apparent in the CM response as harmonic distortion (Calloway et al. 2014).

For analyzing CAPs, the recording signal was filtered off-line between 0.2 and 2 kHz. The peak to peak amplitude of the filtered signal was computed within the first 5 ms after stimulus onset to describe the first negative to positive component of the CAP (N1 to P1). These computations were performed at all electrode contacts (six channels) separately for all stimulation frequencies (1 to 32 kHz) and at all stimulus intensities (0 to 90 dB SPL). Visual inspection of the responses revealed that at high sound pressure levels (70–90 dB SPL) and at frequencies below 2 kHz, CMs strongly contributed to the peak to peak amplitude. We, therefore, excluded frequencies below 2 kHz from further analyses. All response amplitudes were normalized to the overall maximal amplitude of the particular channel. The CAP threshold at each frequency was defined as the lowest intensity that evoked a response exceeding 5% (method adapted from Phillips et al. 1995). From the recorded CAP data, no positional marker for the CI contacts could be determined.

For the analysis of the CMs, the recorded signal was high-pass filtered off-line from 400 Hz. The hardware implemented low-pass filter at 9 kHz (type: butterworth, roll-off: 12 dB per octave) and reduced the signal amplitudes gradually by a factor of $\approx 4$ between 9 kHz (3 dB reduction) and 18 kHz (12 dB reduction), so that some information can still be gained from the data.

Fig. 3. By recording and processing intracochlear signals (electrocochleography [ECochG]) generated in response to acoustic stimulation (raw signal, A), compound action potentials (CAPs, black), cochlear microphonic potentials (CMs, dark gray), and summatting potentials (SP, light gray) could be identified as main underlying components resolved by appropriate filtering (B). (red bar: stimulus duration; stimulation frequency: 8 kHz; stimulation intensity: 90 dB SPL).
collected above 9 kHz. Beyond 18 kHz, CMs are only evoked at sound pressure levels exceeding 90 dB SPL (Schmiedt & Zwislocki 1977) in any case. The root mean square (rms) of the CM amplitude was analyzed in a time window between 10 and 40 ms after stimulus onset, thus, avoiding an influence of the stimulus ramp. We analyzed the data from all recording channels for stimulation frequencies between 4 and 32 kHz (12 steps per octave) at five suprathreshold sound levels (50–90 dB SPL, 10 dB per step). For each recording channel, we normalized the CM amplitudes to the overall maximum and compared the average response at all stimulation frequencies, presented at 70 dB SPL. The CM amplitude variation with stimulus frequency did not follow a simple pattern, and a tuning curve could not be reliably determined. Therefore, the first frequency at the high-frequency edge of the response area that did not evoke above average CM amplitude and bordered at least four subsequent lower stimulation frequencies with above average responses was defined as CM corner frequency ($F_c$; Fig. 7C). We decided on this approach based on the assumption that in a monopolar recording configuration, the recorded signal will become small when it originates at a place between the intracochlear recording electrode and the retroauricular (“far basal”) reference electrode (Chertoff et al. 2012), that is, at or shortly behind the recording electrode. Referencing amplitudes to the average yielded more consistent results than normalization to maximum or minimum values or when defining a fixed threshold value (data not shown). The $F_c$ was subsequently used as a marker for the intracochlear position of the recording contact.

To analyze the SP, we applied first order polynomial smoothing (Savitzky–Golay smoothing filter) with a 5 ms window to the recording signal. This only eliminated high-frequency components from the signal, while having little effect on minima, maxima, and transitions as compared to standard finite impulse response filters. Additionally, the 10 Hz high-pass filtering during signal recording did continuously attenuate the amplitudes of d.c. signal components and, thus, the signal shape. Therefore, a drop of the SP amplitude over time was apparent, and the SP amplitude was determined at the maximum immediately after the first flank in a time window 5 to 20 ms after stimulus onset. We used the same frequency and intensity sampling as for CMs, and the calculation of the position marker (turning frequency, $F_t$) was performed analog to the $F_c$ for CM.

Response thresholds for both CM and SP were determined upon visual inspection. The electrophysiologically determined frequency response maps along the CI according to the positional markers ($F_c$ and $F_t$) were compared to the frequency progression derived from the Greenwood function (Greenwood 1990; $F_g$). Thereby, the lowest frequency (most apical contact) was used as point of origin, and further frequencies were calculated based on the slope of the Greenwood function (2.59 mm/octave). Data were tested for normal distribution before statistical analysis using a Kolmogorov–Smirnov test for normal distribution, two-tailed Wilcoxon signed-rank test: W: 13.5; p: <0.05). The click-evoked CAPs before and after cochleostomy were statistically tested using a two-tailed Wilcoxon signed-rank test: W: 13.5; p: <0.05). The click-evoked CAPs had a median threshold of 30 dB (Fig. 4), despite higher sensitivity compared to ABRs. A substantial loss of auditory sensitivity because of implantation was further supported by the elevated audiograms of unusual form analyzed below.

In intracochlear recordings, a dependency of the ECoG amplitude on recording position and stimulation frequency was apparent mainly in SPs and CMs (Fig. 5). The relation between these measures is analyzed thoroughly in the following paragraphs.

**RESULTS**

**General Findings**

Overall, 20 cochleae were successfully implanted with CIs (six contacts) through a cochleostomy. The click-evoked ABR thresholds before cochleostomy (Fig. 4) demonstrated a good hearing sensitivity at the beginning of the experiment comparable to normal hearing animals in other studies in our lab. After cochleostomy, the thresholds were slightly but significantly elevated (Fig. 4, postoperative ABR failed the Kolmogorov–Smirnov test for normal distribution, two-tailed Wilcoxon signed-rank test: W: 13.5; p: <0.05). The click-evoked CAPs had a median threshold of 30 dB (Fig. 4), despite higher sensitivity compared to ABRs. A substantial loss of auditory sensitivity because of implantation was further supported by the elevated audiograms of unusual form analyzed below.

**CAP Measurements**

Comparing the CAP recordings of the most apical CI electrode (channel 1) to the most basal electrode (channel 6), we found only minor differences in individual recordings.
The response amplitudes and threshold levels were nearly invariant between recording electrodes. On average, the apical channel had slightly higher thresholds (at the boundary of the detection range) than the basal channel for frequencies between 11.3 and 32 kHz. However, these differences between channels were not significant (N = 18; two-way repeated measure ANOVA: no interaction; df\text{channels}, 5; df\text{frequencies}, 20; F\text{channels}, 1.37; F\text{frequencies}, 130.44; p\text{channels}, 0.23; p\text{frequencies}, <0.001). At 70 dB SPL, higher CAP amplitudes were recorded at the most basal electrode at 19 and 22.6 kHz, but the difference was not significant (two-way repeated measure ANOVA: no interaction; df\text{channels}, 5; df\text{frequencies}, 20; F\text{channels}, 1.89; F\text{frequencies}, 152.1; p\text{channels}, 0.093; p\text{frequencies}, <0.001). Because of the overall small effect, no gradual change of CAP threshold and amplitude could be observed between neighboring channels; rather, the differences became apparent only when comparing the electrodes furthest apart. While some position sensitivity can be assigned to CAP recordings, it is not distinctive enough to identify the exact position of the electrode relative to the cochleotopy.

CM Measurements

In contrast to the CAP measurements, considerable position-dependent differences were obvious in the CM recordings (Fig. 7). While multiple local amplitude minima and maxima were distributed along the frequency range studied, the $F_c$ shifted with the recording position (Fig. 7A, B). The CM amplitudes at stimulus levels above 40 dB SPL$_{rms}$ were large enough to make the upper frequency limit discernible, despite the substantial effect of the hardware low-pass filter above 9 kHz. This shift of the upper CM corner frequency ($F_c$) was systematic across all ears studied, however, to various degrees (Fig. 7C). $F_c$ differed significantly between contacts 2 through 6 (Fig. 7D; N=18; one-way ANOVA: df, 5; F, 27.8; p, <0.001; Iglewicz and Hoaglin outlier test, z, ≥3.5; post hoc one-sided, paired $t$ test excluding outliers: df, ≥16; t, <−2.12; p, <0.025). Compared to the slope of the frequency progression calculated from the Greenwood function (2.59 mm/octave), the slope of the $F_c$ shift of the CM was shallower (median $F_c$: apical frequency, 7.13 kHz; basal frequency, 12.7 kHz; distance, 3.5 mm; octaves, 0.83; slope$_{median}$, 4.20 mm/octave; mean $F_c$: apical frequency, 7.47 kHz; basal frequency, 12.22 kHz; distance, 3.5 mm; octaves, 0.71; slope$_{mean}$, 4.93 mm/octave), especially between channels 4 through 6 (arrow heads, Fig. 7D).

SP Measurements

In contrast to the multiple local amplitude minima and maxima of the CMs, the SPs were more “tuned” to a certain frequency (Fig. 8A, B). Typically, the amplitude sharply dropped off at high frequencies, while it trailed off less steeply at the low frequency end of the response. For stimulation at high sound levels, the response maxima were found at frequencies approximately 1/2 octave below the frequency with the lowest response threshold. Both lowest thresholds and response maxima shifted gradually from low frequencies at the most apical contact to high frequencies at the most basal contact. This frequency shift was consistently observed in all cochleae (70 dB SPL; Fig. 8C). At a stimulation level of 70 dB SPL$_{rms}$, often multiple response maxima were observed, especially at low frequencies. We, therefore, introduced the $F_t$ as cochleotopic position criterion (see methods section for details). The $F_t$ defined the sharp upper boundary...
of the SP frequency response area. The difference between \( F_t \) at neighboring recording channels was significant for all channels (Fig. 8D; \( N = 18 \); one-way ANOVA: \( \text{df}, 5; F, 42.18; p < 0.001 \); Iglewicz and Hoaglin outlier test \( z \geq 3.5 \); post hoc one-sided, paired \( t \) test excluding outliers: \( \text{df}, \geq 12; t, < -3.28; p, < 0.005 \)).

The slope of the SP turning frequency progression along the electrode (median \( F_t \): apical frequency, 8.98 kHz; basal frequency, 22.63 kHz; distance, 3.5 mm; octaves, 1.33; slope median, 2.62 mm/octave; mean \( F_t \): apical frequency, 8.93 kHz; basal frequency, 22.43 kHz; distance, 3.5 mm; octaves, 1.33; slope mean, 2.62 mm/octave) is also largely consistent with the expectation based on the Greenwood function (2.59 mm/octave).

Comparison of CM and SP Measurements at Increasing Sound Levels

A comparison of the frequency response areas of the SP and the CM, measured at sound levels between 0 and 90 dB SPL and 1 to 32 kHz with only 4 steps per octave, revealed a more stable pattern of the SP responses with sound level, as compared to the CM (Fig. 9). The upper frequency limit of the SP was steep and shifts shifted only little with increasing sound level at all recording channels, while the amplitude maxima showed a shift toward lower frequencies at high sound levels. The upper frequency limit and the \( F_t \) shifted systematically from high frequencies at the basal recording channels to low frequencies at the apical channels in individual recordings (Fig. 9A), as well as in the grand mean (Fig. 9B). In comparison, the CM responses were more complex and only discernible at high stimulation levels. Below 2 kHz, a response appeared to be present at all recording channels, and above 2 kHz, the amplitudes varied in a complex manner with recording position. The \( F_c \) and the upper frequency limit did not shift progressively from apical to basal recording positions in each individual example (Fig. 9A) but did so in the grand mean (Fig. 9A). Overall, the frequency shift along recording channels was less pronounced than that for the SP.

Evaluation of Histology

In addition to the in vivo ECochG recordings, we determined the cochlear position of the electrode contacts in five cases by microgrinding and subsequent three-dimensional reconstructions (Fig. 2, Materials and Methods). The data obtained from the three-dimensional reconstructions after microgrinding are detailed in Table 1. Based on the Greenwood function for the guinea pig cochlea, we calculated the frequency positions of the contacts relative to the length of each cochlea. The calculated cochleotopic frequency progressions along the cochleae of the five cases are shown in Figure 10A. The frequency positions of the implant varied at maximum by about 1/4 octave, and this corresponds to approximately 650 \( \mu \)m intracochlear distance, close to the actual distance between neighboring CI contacts in
The good estimate pointed to a relatively stable implantation procedure (position of cochleostomy, insertion depth of the implant). The frequency positions determined from histological data (average reconstructed apical position, 8.7 kHz; average reconstructed basal position, 26.3 kHz; distance, 3.5 mm; octaves, 1.59) correlated well with those determined from the SP turning point of the ECochG recordings (linear regression, \( R^2 = 0.898 \); Fig. 8B). Only 2 data points deviated more than 1/2 octave from the ideal linear regression. The average histologically reconstructed frequency slope along the CI (2.20 mm/octave) was steeper than the slope determined from the SP shift (slope\(_{\text{median}} = \text{slope}\(_{\text{mean}} = 2.62 \text{mm/octave}\)). Histological results did not differ significantly from electrophysiological results (two-sided, paired \( t \) test: df, 29; \( t \), 0.521; 2-sided \( p \), 0.606).

**DISCUSSION**

The aim of this study was to determine the suitability of different intracochlear potentials as markers for the cochleotopic position of a CI. We showed for the first time that SPs can serve as reliable markers for a functional positioning of the CI electrodes in cochleae that are functioning or partially impaired. The positional information had an estimated accuracy of ±1/4 octave in the guinea pig when compared with histological reconstructions. This corresponds approximately to the distance between neighboring CI contacts. The general feasibility of intraoperative electrophysiological recordings in human patients has already been shown (Calloway et al. 2014; Campbell et al. 2015, 2016; Dalbert et al. 2015a; Acharya et al. 2016), even for cochleae with substantial levels of sensorineural hearing loss (Choudhury et al. 2012; Calloway et al. 2014; Fitzpatrick et al. 2014).

Our data showed that a CI electrode insertion in normal-hearing animals may cause threshold elevations at frequencies below 19 kHz, along the CI tip and probably extend apically to it. It was detectable in CAP audiograms. Multiple reasons for the threshold shift seem to be plausible. Damage could be either because of mechanical damage during CI insertion and pressure waves caused by the insertion or loss of perilymph (Miranda...
et al. 2014). Also a change in the mechanical properties of the cochlea in the presence of a CI needs to be considered as potential cause for a threshold elevation (Greene et al. 2015). Data from another set of guinea pig experiments (Andrade, Helms- taedter & Baumhoff, unpublished data) indicated that the hearing loss occurred primarily when the implant was fully inserted. A possible explanation for these findings might be a piston effect associated with an occlusion of the Scala tympani by the fully inserted implant and the concurrent (transient) increase in pressure, similar to descriptions in human implantations (Mit tmann et al. 2016; Todt et al. 2016). Preservation of hearing in cochlear regions apical to the CI rises to large importance in human CI patients with residual low-frequency hearing (Miranda et al. 2014) and the possibility of causing trauma during insertion even without direct mechanical contact with the basilar membrane.

The auditory nerve carries the most reliable information about the active frequency regions of the cochlea. Yet the electrode position in the cochlea relative to the active region could not be determined based on CAP recordings in the present study. The CAP recorded at the RW represents a far field response of synchronous activity of auditory nerve fibers (Eggermont 1976a; Rattay et al. 2013). There is evidence that the first negative (N1) and positive (P1) components are a result of the auditory nerve passing through the dura mater at the internal auditory meatus (Brown & Patuzzi 2010). This renders the CAP unsuitable as an intracochlear position marker because near-field effects contribute only little to the overall response amplitude (Brown & Patuzzi 2010). The CAP could, however, be suitable for a functional assessment of the physiological state of the cochlea (Adunka et al. 2010; Campbell et al. 2010, 2016, Choudhury et al. 2011, 2014; DeMason et al. 2012). The small differences observed along the electrode positions were all recorded from within the large basal turn of the guinea pig cochlea. Since we used rise/fall times of 5 ms for our stimuli, we might be unable to see some effects that occur close to the CAP response threshold. Faster rise/fall times and insertions into more apical positions might lead to more pronounced differences of CAP amplitudes between high frequencies in the base and low frequencies at the apex, but the CAP in human recordings is often small with a low signal to noise ratio, making it not suited as a theragnostic tool.

The CM is commonly assumed to be dominated by current flow through the outer hair cells (OHCs) (Dallos 1983; Patuzzi et al. 1989; Cheatham & Dallos 2000; Withnell 2001). Overall,
CMs have been shown to be a more sensitive marker of cochlear trauma local to the recording electrode than CAPs in animals with normal auditory function (Adunka et al. 2010; Campbell et al. 2010; Choudhury et al. 2011, 2014). Furthermore, changes in response strength have been observed depending on the position of the recording electrode (Campbell et al. 2010; DeMason et al. 2012; Calloway et al. 2014). Abrupt reductions of amplitudes across multiple frequencies were interpreted as a physiological marker of trauma inducing interaction with cochlear structures, while gradual and frequency-dependent changes of amplitude were interpreted as positional markers (DeMason et al. 2012; Choudhury et al. 2014). Because of the simultaneous six-channel recordings at fixed intracochlear positions in the present study, as opposed to a stepwise insertion of a single electrode, it was possible to relate the interchannel differences solely to position. Thus, any global influence from changes in the physiological state or mechanical interference between implant and cochlea, not fully controllable during insertion experiments with sequential recordings at different insertion depths, could be excluded. The maximum amplitude was ambiguous as a marker of cochlear position because there were typically several amplitude peaks at different frequencies. These peaks overlapped between recording electrodes.

**Fig. 9.** Summating potential (SP) responses depend more distinctly on recording position than CM responses. The $F_t$ of the SP (red, left side of the plots) shifts systematically from high frequencies (ordinate) at the basal recording contacts (channel 6, top) to lower frequencies for the more apical recording contacts (channel 1, bottom), both in an individual example (A) and the grand mean (B). The $F_t$ stays stable for a broad range of sound levels (abscissa). The frequencies noted in the plot (solid black lines) are the $F_t$ values determined at 70 dB SPL (dashed black line) from recordings with higher frequency resolution (Fig. 7). In contrast, the CM (green, right side of the plots) is only measurable at high sound levels (abscissa mirrored for better comparison). The amplitude distribution along frequencies is more complex, with strong responses below 2 kHz that are similar for all recording contacts and responses above 2 kHz that are variable between contacts. The $F_t$ does not shift systematically along the recording contacts in the individual example (A) but shows position dependence in the grand mean (B). Furthermore, the high-frequency edge of the CM response area shifts toward lower frequencies at high sound levels, while this effect is negligible for SP responses. Color scales of the normalized response amplitudes are indicated at the bottom of the figure.
of CMs or slightly further basal. While certainly carrying some bias for basal locations, such an estimate would also prevent a penetration into the functional, more apical, cochlear regions in a clinical application. A more problematic issue was the shallow CM corner frequency progression in comparison to the estimate from the guinea pigs Greenwood function (slope_\text{median}, 4.20 mm/octave and slope_\text{mean}, 4.93 mm/octave compared to 2.59 mm/octave; Greenwood 1991). While the influence of the 9 kHz low-pass filter and the resulting reduction in recording amplitudes is part of the explanation, a substantial deviation from the ideal progression is already apparent at the three most apical contacts at frequencies that were largely unaffected by the filter. The frequency increase between the most apical contact and contact 2 shows a strong overlap and no significant difference in frequency, even though the hardware filter has no effect in this low-frequency range. A possible explanation for this observation is that fast phase rotations of the large hair cell responses occur close to the cochleotopic frequency position. These variations in phase result in a spatial cancellation of the CM (Avan et al. 2013). At the position of the recording electrode, the CM, therefore, becomes small or is even absent and cannot be used as a positional marker. An upper frequency limit exists for each recording position, dividing apical from basal CM responses (Dallos 1972). Taken together, these considerations indicate that the CM alone is not suited as marker for the cochleotopic position of a CI.

The SP is the d.c. component of ECochG and has received less attention as potential marker for cochlear damage or electrode position in earlier studies because of the complexity of its composition and multiple contributing generators (Dallos 1973); usually inner hair cells (IHCs) are assumed to be the main generators for the SP (Zheng et al. 1997; Durrant et al. 1998), but depending on recording position, OHCs also contribute to the SP (Dallos 1986). The ratio between these contributing currents is subjected to a frequency-dependent shift (Cody & Russell 1985; Russell et al. 1986), further described below. While we cannot exclude a neural d.c. contribution by the auditory nerve sustained potential (Forgues et al. 2014), we did not find any indication for diminished amplitudes in regions where the CAP thresholds were affected after the CI insertion. This indicates that we were most likely recording d.c. potentials from intact hair cells, rather than the nerve. It has also been hypothesized that the SP is a consequence of an unbalanced CM because of the nonlinear vibration of the basilar membrane at moderate to high stimulus intensities (Johnstone & Johnstone 1966; Honrubia & Ward 1969a, b; Dallos 1973; Gibson 2017). Therefore, the amplitude maximum of SP could originate remote from the recording site similar to the CM described.

TABLE 1. Some basic histological measures reconstructed from microgrinding of five guinea pig cochleae

<table>
<thead>
<tr>
<th>Case ID</th>
<th>img. No.</th>
<th>length (mm)</th>
<th>err_mean (%)</th>
<th>err_max (%)</th>
<th>turns</th>
<th>prop_turns (%)</th>
<th>length (mm)</th>
<th>ins.dep. (mm)</th>
<th>ins.a. (°)</th>
<th>el.cov. (%)</th>
<th>pC6 (%)</th>
<th>fC6 (kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28J14re</td>
<td>25</td>
<td>15.31</td>
<td>17</td>
<td>31</td>
<td>3.5</td>
<td>4.8</td>
<td>18.78</td>
<td>5.01</td>
<td>212.5</td>
<td>23.3</td>
<td>90.8</td>
<td>27.9</td>
</tr>
<tr>
<td>28J14li</td>
<td>21</td>
<td>14.22</td>
<td>8</td>
<td>17</td>
<td>3.25</td>
<td>6.1</td>
<td>16.29</td>
<td>4.45</td>
<td>196.9</td>
<td>24.8</td>
<td>89.8</td>
<td>26.7</td>
</tr>
<tr>
<td>16J14li</td>
<td>22</td>
<td>16.08</td>
<td>9</td>
<td>27</td>
<td>3.25</td>
<td>6.1</td>
<td>18.59</td>
<td>5.12</td>
<td>209.9</td>
<td>22.5</td>
<td>87.0</td>
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</tr>
<tr>
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<td>23</td>
<td>15.35</td>
<td>13</td>
<td>38</td>
<td>3.25</td>
<td>6.1</td>
<td>18.41</td>
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<td>192.1</td>
<td>20.8</td>
<td>88.0</td>
<td>24.3</td>
</tr>
<tr>
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<td>21</td>
<td>14.43</td>
<td>20</td>
<td>44</td>
<td>3.25</td>
<td>6.1</td>
<td>18.38</td>
<td>4.50</td>
<td>197.6</td>
<td>21.6</td>
<td>81.7</td>
<td>29.2</td>
</tr>
</tbody>
</table>

err_cov. indicates proportional cochlear coverage with the implant; err_max, maximum error; err_mean, mean error; fC6, theoretical frequency at contact 6 based on Greenwood-function (Greenwood 1991); img., number of microgrinding images in the stack; ins.a., insertion angle; ins_dep., corrected insertion depth of the electrode; length, corrected length; lengthr, reconstructed length; pC6, position of contact 6 relative to cochlear length w/o hook region; prop., missing proportion of the cochlea based on number of turns; turns, number of reconstructed turns.

Fig. 10. The frequency positions of the implant’s contacts were reconstructed from microgrinding images according to the Greenwood function (Greenwood 1991) for the five cases presented in Table 1 (A). We observed an overall good correlation between the electrophysiologically summing potentials [SP] and histologically obtained frequency–position estimates (B). Of all data points, 52% (13) lay within 0.25 octaves from the ideal correlation, another 40% (10) within 0.5 octaves, and only 8% (2) lay outside of this range. The dashed red line indicates the linear regression for all data points (R² = 0.898). Frequencies at the apical contacts tend to be lower in the histological reconstruction compared to the electrophysiological estimate, while they tend to be higher at the basal contacts. Five data points, identified as outliers in the electrophysiological analysis, were excluded from the plot (see Fig. 7). (Data point numbers refer to contact/channel numbers, cochleae: n = 5; data points analyzed: n = 25; refer to text for full statistics.)
above. In the data presented here, we did not see such similarities between SP and CM. Furthermore, the frequency tuning of the SP maximum varied little with stimulus intensity and, most importantly, \( F_t \) used as positional marker in this study was very frequency stable.

Regardless of the actual composition of the SP, the strong IHC contributions and sharp frequency tuning (Dallos 1973; Cheatham & Dallos 1984) make it an interesting option for an electrophysiological positional marker. We also did find examples of sharply tuned SPs in well-functioning regions of the cochlea. The highest SP amplitudes were typically recorded to frequencies 1/2 octaves below the tip of the tuning curve. This can largely be attributed to the shift of the BM's displacement maximum toward more apical positions with lower characteristic frequencies with increasing stimulus intensities (Johnstone et al. 1986; Ruggero & Rich 1991). Additionally, the intracochlear current flow (Dallos 1972) and a d.c. contribution from CM distortions (Johnstone & Johnstone 1966; Honrubia & Ward 1969a, b) could add to the shift. Furthermore, a shift between OHCs and IHCs as generators for the SP from apex to base has been described by Cody and Russell (1985). This might contribute to a frequency shift from the excitation maximum, marked by strong IHC contributions, toward the place with the strongest activity of the OHC to amplify softer stimulus components. The experiments of the present study did not indicate a gradual negative-to-positive transition of the potential from low to high frequencies as described in earlier studies (Dallos 1972, 1986). Instead, the amplitude of the SP sharply dropped off. The reason for this is likely the far remote position of the reference electrode in the skin over the lateral masseter muscle.

Dallos et al. described spatially narrow positive components of the SP dependent on stimulus frequency in recordings from the guinea pig cochlea (Dallos et al. 1970; Dallos 1972). It was described as the average of the potentials of the Scala vestibuli and the Scala tympani. Separate recordings from both scalae did not result in the same positional selectivity. In the present study, we did not find maximum positive components of the SP as spatially selective as described by Dallos et al. (1970). This might be because of the use of a different recording setup with a remote position of the reference electrode and large differences in recording impedances (Dallos 1970, \( \approx 10 \) MOhm, method described in Tasaki et al. 1952; present study \( \approx 5 \) kOhm), possibly affecting the range of spatial integration, similar to what has been described for local field potentials in the brain (Nelson & Pouget 2010). Yet the overall pattern of positive SPs had a high spatial selectivity, especially when considering the high-frequency edge of the recorded frequency response areas. The slope of positional estimate based on \( F_t \) calculated from the electrophysiological data of the present study (slope = \( \text{slope}_{\text{median}} = 2.62 \text{mm/octave} \)) compared well to the estimate based on the Greenwood function of the guinea pig (2.59 mm/octave; Greenwood 1991). Using the upper frequency limit of the SP as marker of the intracochlear recording position, therefore, seems feasible.

Compared to the SP tuning, the tuning of CMs is relatively broad (Schmiedt & Zwislocki 1978; Cheatham & Dallos 1984; Ayat et al. 2015). The SP tuning curves are frequency stable over a broad range of intensities and corresponds to the place of maximum BM displacement (Cheatham & Dallos 1984), while maximum amplitude of the CM tuning shifts to more apical positions with increasing intensities (Honrubia & Ward 1968). This could also be shown in the present study. The frequency shift of the CM occurs because it is generated by OHC that amplify small BM displacements (Patuzzi et al. 1989), which are at high-frequency locations caused by low-frequency traveling waves passing the more apical positions (“tail” of the neuronal tuning curve; Davis 1983). With that, the \( F_t \) of the CM is not reliable as positional marker at 70 dB SPL, as it will be underestimated because of the shift of tuning, whereas the \( F_t \) of the SP is spatially more restricted and stable.

The histologic preparation of harvested guinea pig cochlea allowed an adjustment of the Greenwood function to the actual length of the cochlea investigated, instead of using a species-specific average. The microgrinding technique (Stöver et al. 2005) has the advantage of preserving the soft tissue elements, allowing a visualization of all cochlear structures and the CI. In a three-dimensional reconstruction from the images taken during the micro-grinding process, errors from slight variations in abrasions between the images needed to be compensated. This led to a less exact estimation of cochlear length and the positions of CI contact within the cochlea (see Materials and Methods). Therefore, the variance in the frequency slope across animals (compare Fig. 8) can either be because of anatomical variabilities or be because of reconstruction errors. Overall, the slope of 2.20 mm/octave is slightly steeper than the literature value of 2.59 mm/octaves. In comparison, the individual electrophysiological data and the respective estimates of the histologic reconstruction are closely correlated, and the deviation is only moderate. Overall, the reconstruction of the CI electrode positions within the cochlea according to the Greenwood function resulted in a similar frequency range as that estimated from the electrophysiological data. Therefore, the histologic reconstruction validates the frequency range of the electrophysiological data, while the actual tonotopic progression was better represented by the electrophysiologically obtained median frequency values per contact. This possibly results from the higher sample size of the electrophysiological data. Additionally, a methodological difference between measuring the Greenwood function and our recordings needs to be considered. While the Greenwood function is often acquired by tone on tone masking at moderate to low sound levels (Greenwood 1991), most of the data of the present study were obtained at a fixed sound level of 70 dB SPL. We did so to stay close to a potential clinical application, where time restrictions during surgery call for a fast method, and multiple sound levels cannot be accessed. Recordings across a broad range of sound levels showed the SP to be a very stable measure in the present study. Loss of hair cells (IHCs as well as OHCs) leads to diminished SP amplitudes (Durrant et al. 1998), while other pathologies can also lead to an increased SP amplitude (Yamasoba et al. 1993). Therefore, the usefulness of the SP as positional measure in human CI recipients with various etiologies is unclear needs further investigation. Additionally, the frequency range we recorded in the guinea pig (>4 kHz) is much higher than the range of hearing preservation in human CI recipients (<1 kHz). However, SP could be recorded for frequencies as low as 300 Hz in humans (Ferraro et al. 1994). We, therefore, consider the SP, possibly in a yet to be established combined measure with the CM and CAP, promising as positional marker when recorded intraoperatively from CI contacts.
CONCLUSION

This study provides a proof of principle for the usability of intracochlear recordings as marker for the tonotopic position of the CI contacts. While CAPs appeared to be sensitive markers for the physiological state of the cochlea, reflecting cochlear damage, CMs and SPs provide information on the frequency position of the electrode contacts in the cochlea. The CMs were less reliable with shallow frequency slope compared to the Greenwood function, especially at the three most apical contacts. The frequency slope along all six CI contacts based on SP closely resembled the expectation from the Greenwood function. This makes the SP a good candidate for intracochlear position monitoring, possibly in combination with an analysis of the CM and the CAP for monitoring cochlear health. The results of the present study are promising and could pave the way for an intraoperative monitoring technique that assists the surgeon and helps to preserve residual hearing in human subjects. Our data show the feasibility of recordings from the contacts of a CI itself and show the need for access to unfiltered signals recorded over the CI.

ACKNOWLEDGMENTS

The authors thank Dr. W.S. Konerding for comments on an earlier version of the manuscript and help with the statistical analysis. We are also grateful to MED-EL Comp. (Innsbruck, Austria) for providing the custom-build research cochlear implants for this study. This work was supported by the German Research Foundation Deutsche Forschungsgemeinschaft (DFG) Cluster of Excellence Hearing4All, Hannover, Germany (DFG EXC 1077). The authors have no conflicts of interest to disclose.

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Received November 29, 2016; accepted October 8, 2017.

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