INTRODUCTION
Electrode array insertion during cochlear implant (CI) surgery usually causes some degree of trauma to the delicate structures of the cochlea. This trauma is known as electrode insertion trauma (EIT) and may initiate mechanisms that can lead to ganglion cell death, necrosis, and apoptosis. Histology of temporal bones from unilaterally implanted patients reveals a modest decrease in the total number of spiral ganglion cells in the implanted ear compared with the non-implanted ear, which indicates that EIT has caused degeneration of these cells. Any damage to the functional cochlear structures of the inner ear can lead to degeneration of neural tissue, which is the target of electric stimulation provided by a CI. Therefore, the goal of CI surgery is to minimize the extent of EIT. Moreover in patient with partial deafness, hearing preservation is a fundamental component for combining electric hearing with acoustic hearing in the same ear [1].

The development of less traumatic electrodes and improved surgical techniques have resulted in increasing rates of preservation of low-frequency acoustic hearing in the implanted ear - after shallow [2], medium [3,4], and deep insertion of electrode arrays designed for lateral wall placement [5]. Due to the general assumption that the amount of EIT significantly correlates with the level of postoperative hearing preservation, the extent of preservation is believed to serve as a good indicator of the magnitude of EIT [5]. However, it is not known how EIT affects cochlear health and function beyond that which is revealed by the audiogram. Since 1935, when Fromm, Mylen, and Zotterman measured the cochlear potential in two subjects, electrocochleography (ECoG) has been considered an excellent measure of cochlear function. Extracochlear ECoG is a recording of sound-evoked cochlear and auditory nerve responses from the round window (RW), promontory, eardrum, or external ear canal [6]. During cochlear implantation, RW ECoG has been used to measure co-
chlear function to predict postoperative hearing preservation [7-10] and speech perception outcomes [11-14]. To predict postoperative hearing preservation, extracochlear ECoG can also be recorded intraoperatively from the promontory [10, 13] or from the stapes [16].

The extracochlear ECoG recorded from CI users, however, may not give sufficient information on frequency or location dependent events within the cochlea. Furthermore, this technique is invasive, involving the use of an external electrode attached to an external evoked potential (EP) device that can be difficult to perform after cochlear implantation. For this reason, this study involves a collaboration with Med-El Corp., which allowed for ECoG potentials to be measured via the CI electrodes across various locations within the cochlea. The feasibility of such recordings was presented at the XXXII World Congress of Audiology in 2014 [17]. Furthermore, the extracochlear ECoG has previously been performed across other studies, using either a flexible intracochlear recording electrode [18] or a CI electrode contact as the recording electrode [10, 19-23]. These preliminary studies showed that the intracochlear ECoG could be directly recorded from the CI electrode in CI recipients with residual hearing. This may prove to be useful for intraoperative and postoperative monitoring of cochlear health.

However, limited information is available on the optimized recording parameters or how the ECoG potentials depend on the location within the cochlea for intracochlear ECoG responses and stimulation frequency. This study focuses on the recordings of cochlear microphonic potentials (CM) from different locations within the cochlea of CI users with deeply inserted electrodes. The aims of this study were to identify: 1) the most sensitive frequency to record the CM in subjects with a wide range of hearing abilities before and after receiving a CI, and 2) the optimum location within the cochlea to record intracochlear potentials.

MATERIALS AND METHODS
The ethical committee approved the study protocol. Each prospective subject was given an informed consent form that explained the purpose and procedures involved in the study. If the patient agreed to participate, the informed consent was signed. The procedures followed were in accordance with the ethical standards of the Helsinki Declaration.

Sixteen subjects (eight females and eight males) with varying degrees of hearing abilities, both before and after receiving their CI, were selected for the study. The subjects were implanted with the following electrode arrays: Flex 20 (n=1), Flex 24 (n=7), Flex 28 (n=1), Flex Soft (n=5), Medium (n=1), and Standard (n=1). The subjects received either a Pulsar, Concerto, or Sonata CLs, Med-El Corp. Each subject was implanted via the round window insertion technique [3, 22]. The mean age was 48 years and 4 months at the time of surgery (range 20-68 years). The mean time of measurements was 13 months (range 2-91 months after the CI surgery). The preoperative audiogram was obtained 1-4 weeks prior to receiving the cochlear implantation. The postoperative audiogram was obtained at the time of this study.

Recordings of Intracochlear ECoG
Intracochlear acoustically evoked potentials were recorded from the CI electrodes. Patients were positioned in a comfortable semi-lying position. Inserts were placed into their implanted ear. The inserts were connected to the Nicolet EDX (Natus Corp., Middleton, USA) that was used for acoustic stimulation. The Research Evoked Potentials (EP) software, (Med-El Corp., Innsbruck, Austria) was run from a PC communicating with the MAX Interface (Med-El Corp. Innsbruck, Austria). The MAX interface communicated via an external coil connected to the CI. This is possible when the external coil is placed on the CI. When the recording was initiated, the MAX interface triggered the Nicolet EDX that acoustically stimulated the patient. The TIP 300 inserts were used for the acoustic stimulation calibrated TIP.

For the acoustic stimuli, either the tone pips of frequencies 250, 500, 1k, 2k, and 4 kHz or 1 ms click were used. The duration of the tone pips of 250, 500, 1k, 2k, and 4 kHz were 12 ms (3 cycles), 8 ms (4 cycles), 5 ms (5 cycles), 2.5 ms (5 cycles), and 1.25 ms (5 cycles), respectively. The duration of the tone pips was chosen to be sufficiently long to identify the CM in the response. The advantage of evaluation of CM response is that only single stimuli polarity is sufficient [24]. Therefore, each of the stimuli was of condensational polarity.

The Research EP software allowed for the recordings window to be increased up to 20 ms. Depending on the stimuli used, the recording window was increased from 5 to 20 ms. For instance, for the tone pips recordings at 250 Hz pips, the time window was set from 9 to 20 ms, while for the tone pips of 4 kHz, the time window was set to 5 ms. The minimum number of averages was set to 100. Depending on the signal to noise ratio (SNR), the number of averages varied from 100 to 300 (i.e., larger signals require fewer averages and small signals require more averages).

During our feasibility phase, several control measurements were performed. Control tests were conducted to evaluate the possibility of electromagnetic coupling between the insert earphones placed in the ear canal and the implant electronics or possible mechanical vibration of the intracochlear electrode array as proposed by Koka and his colleagues [31]. Neither electromagnetic nor mechanic distortion was present at the maximum calibrated levels of inserts.

An electromagnetic field created by the sound generator largely rules out the possibility of an artifacts within the calibrated range of stimulation, as the curves are subject to a time delay attributable to the speed of sound propagation (Figure 1).

Furthermore, to guarantee physiological response, the additional measures were followed: 1) reproducible recordings for the same stimuli conditions; 2) responses decreased with the decreasing stimulating amplitude; 3) responses with the refractory stimulation showed inverted polarity; 4) responses were flat at the minimum stimulating level, and 5) prior measurements inserts were calibrated. An example of recordings to stimulating level change and various stimuli polarity is shown in Figure 2.

The CM was evaluated as a signal of the frequency of the stimulus across a minimum of three repetitions. The peak-to-peak amplitude of this signal ($A_{CM_{pp}}$) was measured at the frequency of the stimuli, and the maximum peak-to-peak amplitude ($A_{CM_{pp}}$) was measured for the 1 ms click signal. The evaluation was performed in time and frequency domain.
Before measuring the intracochlear ECoG, the maximum comfortable level (MCL) was attempted to obtain for each stimulus. All recordings were then performed at the MCL.

For each subject, an unaided preoperative audiogram was performed within four weeks prior to the surgery, and an unaided postoperative audiogram was performed on the day of the study. At our center, the audiometers used are able to stimulate up to 115 dB HL. Therefore, for situations where no acoustic threshold was obtained at 115 dB HL, the threshold was substituted by 120 dB HL. At the time of the study, a postoperative CT scan was obtained for each subject, and the electrode placement was evaluated.

**Statistical Analysis**

Statistical analysis was performed using standard parametric techniques (analysis of variance [ANOVA] single factor test and two factor with replication, and appropriate post hoc test) comparing the mean maximum $A_{CMpp}$ in terms of their dependency on the cochlear location, and the mean maximum $A_{CMpp}$ for tone pips of frequencies 250, 500, 1k, 2k, and 4k Hz along with the mean maximum amplitude $A_{pp}$ for the 1 ms click. A criterion of $\alpha=0.05$. For the two-group comparison of means, a two-tailed t-test was chosen. The criterion for statistical power was $>0.9$.

**RESULTS**

The mean electrode insertion at the tip of the array was 531.6° (from 388° to 750°). The detailed data are in Table 1. Figure 3 provides an example of a CT electrode insertion evaluation.

Figure 4 depicts the mean and standard deviations of the unaided preoperative and postoperative audiograms. Additionally, it includes the mean stimulating levels for tone pips at frequencies 250, 500, 1k, 2k, and 4 kHz. They were 100.8, 105.8, 112.4, 115.4, and 115 dB HL.
respectively. For the 1 ms click, the mean stimulating level and the standard deviation was 105.9±5.1 dB HL. For tone pips with a frequency of 250, 500, and 1 kHz, the MCL was not achieved for two of the subjects. For the 2 kHz tone pip, the MCL was not achieved across four of the subjects. For the 4 kHz tone pip, the MCL was not achieved in eight cases, and for the 1 ms click, the MCL was not achieved in five subjects.

The mean low-frequency pure tone average (PTA) (125-500 Hz) before CI surgery was 52.2 dB HL (from 8.3 to 81.7 dB HL). The mean low-frequency PTA shift after the CI surgery, as recorded at the time of this study, was 9.1 dB HL (range 0-30 dB HL).

It was possible to record the CM amplitudes for every patient across the full tone pips frequency range. Similarly, for the 1 ms click stimuli, it was possible to obtain responses from every subject. Figure 1 depicts an example of these recordings. The thick grey line at the bottom of the figure represents the CM amplitudes for every patient across the full tone pips frequency range.

Table 1. Demographic data of the study subjects. The PTA refers to the average of hearing threshold levels at 125-500 Hz.

| Subject | Sex   | Implant/ Electrode      | Date of CI surgery | Time of study [months after CI implantation] | Degree of electrode insertion [°] | PTA at the PTA pre-op [dB HL] | PTA at the PTA post-op [dB HL] |
|---------|-------|-------------------------|--------------------|-----------------------------------------------|----------------------------------|-------------------------------|--------------------------------|
| S01     | Female| CONCERTO FLEX20         | Aug 2014           | 2                                             | 80                              | 8.3                           | 10.0                           |
| S02     | Male  | SONATA FLEXSOFT         | Mar 2014           | 8                                             | 659                             | 71.7                          | 75.0                           |
| S03     | Male  | CONCERTO MEDIUM         | May 2014           | 6                                             | 589                             | 41.7                          | 71.7                           |
| S04     | Male  | SONATA FLEXSOFT         | Mar 2014           | 9                                             | 565                             | 71.7                          | 75.0                           |
| S05     | Male  | SONATA FLEXSOFT         | May 2014           | 6                                             | 635                             | 65.0                          | 58.3                           |
| S06     | Male  | CONCERTO FLEXSOFT       | Feb 2014           | 9                                             | 515                             | 56.7                          | 56.7                           |
| S07     | Female| CONCERTO FLEX24         | Aug 2014           | 9                                             | 472                             | 25.0                          | 33.3                           |
| S08     | Female| SONATA FLEXSOFT         | Sept 2014          | 2                                             | 729                             | 81.7                          | 81.7                           |
| S09     | Female| SONATA FLEX28           | Sept 2014          | 2                                             | 597                             | 58.3                          | 65.0                           |
| S10     | Male  | CONCERTO FLEX24         | April 2014         | 7                                             | 400                             | 70.0                          | 70.0                           |
| S11     | Male  | SONATA FLEX24           | July 2014          | 4                                             | 470                             | 81.7                          | 83.3                           |
| S12     | Female| CONCERTO FLEX24         | Mar 2014           | 8                                             | 472                             | 51.7                          | 56.7                           |
| S13     | Female| CONCERTO FLEX24         | April 2014         | 8                                             | 503                             | 61.7                          | 75.0                           |
| S14     | Female| SONATA FLEX24           | Sept 2013          | 15                                            | 440                             | 13.3                          | 16.7                           |
| S15     | Male  | PULSAR FLEX24           | Aug 2012           | 28                                            | 480                             | 35.0                          | 76.7                           |
| S16     | Female| PULSAR STANDARD         | April 2008         | 91                                            | 381                             | 41.7                          | 75.0                           |
tom of all recordings shows the duration of acoustic stimulation delivered to the ear canal. The start of the acoustic stimulation includes 0.8 ms delay caused by the inserts.

Figure 5 shows the incidence of locations within the cochlea where the maximum $A_{\text{CMpp}}$ and $A_{\text{pp}}$ amplitudes were recorded. The locations within the cochlea are represented by the degree of electrode insertion. For simplicity, the 0° location is set at the round window. The frequency distribution was calculated according to Stakhovskaya et al. The maximum CM amplitudes may occur at various locations within the cochlea for different subjects.

Figure 6 shows the mean maximum $A_{\text{CMpp}}$ amplitudes to tone pips and $A_{\text{pp}}$ amplitudes to click stimuli in terms of their location within the cochlea. An ANOVA two factor without replication test showed that the locations differed in terms of CM amplitudes for various tone pips and amplitudes of the 1 ms click ($\alpha=0.05$; $p<0.001$; $F=29.80$). Table 2 provides a statistical comparison of the recorded means.

The mean degree of electrode insertion at the maximum $A_{\text{CMpp}}$ and $A_{\text{pp}}$ measured for the tone pips at frequency values of 250, 500, 1k, 2k, and 4k Hz and for the 1 ms click were 312.2 ±198.5, 294.4±182.1, 248.4±181.0, 171.6±149.6, 152.8±129.2, and 295.3±218.2, respectively. The post hoc statistics showed that the mean degree of insertion of maximum $A_{\text{CMpp}}$ amplitude to tone pips and $A_{\text{pp}}$ amplitude to click stimuli were not the same. The deeper into the cochlea the mean maximum CM peak-to-peak amplitude occurred, the lower the stimulating tone frequency was. Figure 7 depicts the individual distribution on the maximum $A_{\text{CMpp}}$ and $A_{\text{pp}}$.

Figure 8 shows the mean maximum $A_{\text{CMpp}}$ amplitudes for tone pips of frequencies 250, 500, 1k, 2k, and 4 kHz and the mean maximum $A_{\text{pp}}$.

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**Table 2.** A statistical data comparison of the means of the locations within the cochlea for the maximum $A_{\text{CMpp}}$ amplitudes of the tone pips at frequencies of 250, 500, 1000, 2000, and 4000 Hz and the $A_{\text{pp}}$ amplitudes of the 1 ms click. T indicates the results of a Student’s t-test, p shows significance level for each comparison.

|        | 500 Hz | 2500 Hz | 4 kHz | 1 ms click | 500 Hz | 2500 Hz | 4 kHz | 1 ms click | 500 Hz | 2500 Hz | 4 kHz | 1 ms click |
|--------|--------|---------|-------|------------|--------|---------|-------|------------|--------|---------|-------|------------|
| $A_{\text{pp}}$ |        |         |       |            |        |         |       |            |        |         |       |            |
| T      | 1.24   | 1.88    | 3.53  | 3.15       | 1.38   | 1.39    | 3.14  | 3.08       | 0.04   | 2.87    | 2.46  | 1.37       |
| p      | 0.23   | 0.08    | 0.003 | 0.007      | 0.19   | 0.18    | 0.007 | 0.008      | 0.96   | 0.01    | 0.03  | 0.19       |
amplitude for the 1 ms click. The recorded amplitudes varied from 10 to 620 µV. The mean peak-to-peak amplitude among all types of stimuli used in patients varied from 19.2 to 536.7 µV. The ANOVA two factor without replication test showed differences in the mean amplitudes (α=0.05; p<0.001; F=13.49). Table 3 provides a statistical comparison of the recorded means. The mean maximum $A_{CMpp}$ amplitudes for tone pips of frequencies 250, 500, 1k, 2k, and 4 kHz and the mean maximum $A_{CM}$ amplitude were 105.1±116.2, 148.2±151.6, 160.7±170.5, 119.9±111.2, and 167.1±170.6 µV, respectively. The data suggest that the highest sensitivity occurs for stimuli 1 ms click, 1 kHz, and 500 Hz tone pips.

### DISCUSSION

One of the goals of this study was to identify the most sensitive stimulus to record CM in CI users. For this reason, we evaluated patients with a wide range of hearing abilities before and after cochlear implantation. It was possible to record the CM amplitudes for every subject across all of the tone pips frequencies or amplitudes for 1 ms click. The data suggest that the highest and therefore most sensitive responses occurred for the 1 ms click, along with the 1k and 500 Hz tone pips. The advantage of the 1 ms click is that it reflects the sensitivity required for patients with low-frequency hearing. In case we are interested in just single frequency stimuli, 1 kHz was selected. Similar observations were seen in the study done by Bester et al. [23].

For our study results, when comparing to the cochlea tonotopy according to Stakhovskaya et al. [25], 250, 500, and 1 kHz tone signals had their most excitable locations at much higher degrees of insertion, with the differences varying from 300° to approximately 100°, respectively. However, no remarkable differences were observed between the most exciting locations for tone signals at 2 or 4 kHz in our results (Figures 5, 6). This may suggest that more mechanisms, including the proven Bekesy theory, are taking place in the cochlea in our study subject. The presence of the electrode array in the cochlea may have influenced the results. Specifically, in many cases, the insertion depth of 250 Hz and 500 Hz was not reached, thus the maximum CM amplitudes are not necessarily highest at the most excitable area in every patient (Figure 4). Campbell and his colleagues previously observed this phenomenon in three patients. However, two subjects tested with 500 Hz tone pips showed reduced CM amplitude on the more basal electrodes than on the apical one, and one subject tested with 1 kHz pips showed the largest CM amplitudes on the most basal electrode. [20]. Similar observations were seen in the study done by Bester et al. [23].

The data suggest that in all subjects in this group, the number of remaining hair cells and neural structures were sufficient to record. The low-frequency PTA at the time of this study varied from 10.0 to 83.3 dB HL (Table 1). At the characteristic frequency of 500 Hz, which is the frequency that is often used to estimate low-frequency hearing, the hearing levels varied from 10 to 100 dB HL. Therefore, this means that the hearing level could be as low as 100 dB HL, and the recordings of CMs of various frequencies could be systematically obtained. Thus, it is possible to record CMs in patients with a very low level of residual hearing. This broadens the application of measurements of acoustically evoked intracochlear potentials to very wide range of CI users. This new application opens up a new field that has the potential to reveal new knowledge on cochlear properties, patients, and CI electrodes.

The data suggest that the average angular insertion depth of the arrays does not necessary lead to the most sensitive stimulus frequency. In our study, the mean electrode array insertion was 531.6° (from 388° to 750°) with the closest apical to the average angular insertion of 250 Hz stimulus frequency. Despite this, the most sensitive stimulus frequency was 1 kHz.

For our study results, when comparing to the cochlea tonotopy according to Stakhovskaya et al. [25], 250, 500, and 1 kHz tone signals had their most excitable locations at much higher degrees of insertion, with the differences varying from 300° to approximately 100°, respectively. However, no remarkable differences were observed between the most exciting locations for tone signals at 2 or 4 kHz in our results (Figures 5, 6). This may suggest that more mechanisms, including the proven Bekesy theory, are taking place in the cochlea in our study subject. The presence of the electrode array in the cochlea may have influenced the results. Specifically, in many cases, the insertion depth of 250 Hz and 500 Hz was not reached, thus the maximum CM amplitudes for the low-frequency tones may have occurred at higher insertion angles. It is of further interest to evaluate this effect further. In addition, there may be “dead-regions” along the cochlea, where there are no hair cells (even though there may still be auditory thresholds), and this too could influence the place at which the maximum CM amplitude is found.

| App | 500 Hz | 1 kHz | 2 kHz | 4 kHz | 1 ms click | 1 kHz | 2 kHz | 4 kHz | 1 ms click | 1 kHz | 2 kHz | 4 kHz | 1 ms click |
|-----|--------|-------|-------|-------|-----------|-------|-------|-------|-----------|-------|-------|-------|-----------|
|     | 0.12   | 0.06  | 0.08  | 0.22  | 0.004     | 0.45  | 0.25  | 0.005 | 0.31      | 0.11  | 0.06  | 0.13  | 0.006     |
| p   | 0.22   | -0.79 | -1.91 | -0.79 | -1.21     | -0.6  | -0.83 | -0.28 | -0.19     | 3.11  | 4.20  | 0.001 | 0.007     |
| p   | 0.12   | 0.06  | 0.08  | 0.22  | 0.004     | 0.45  | 0.25  | 0.005 | 0.31      | 0.11  | 0.06  | 0.13  | 0.006     |
| T   | 1.21   | 1.60  | -1.91 | -0.79 | -1.21     | -0.6  | -0.83 | -0.28 | -0.19     | 3.11  | 4.20  | 0.001 | 0.007     |
CONCLUSION
The measurements of acoustically evoked intracochlear potentials via the location dependent intracochlear electrodes are systematically recordable in a wide range of postoperative hearing abilities of cochlear implantees. Among the tone pips of various frequencies, 1k or 500 Hz appear to be the most sensitive for CI users with low-frequency postoperative hearing.

The most sensitive location within the cochlea to record CM potentials depends on the frequency tone used. The deeper in the cochlea the mean maximum CM peak-to-peak amplitude is, the lower the stimulating tone frequency will be.

Ethics Committee Approval: The study was designed and conducted according to the Declaration of Helsinki, and the study protocol was reviewed and approved prior to the study by the Institutional Review Board at the Institute of Physiology and Pathology of Hearing.

Informed Consent: Written consent was obtained from all subjects participating in the study.

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