Cochlear microphonic potential recorded by transtympanic electrocochleography in normally-hearing and hearing-impaired ears

Potenziale microfonico cocleare registrato mediante elettrococleografia transtimpanica in soggetti normoudenti e in soggetti con ipoacusia neurosensoriale

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Parole chiave
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Summary
The cochlear microphonic is a receptor potential believed to be generated primarily by outer hair cells. Its detection in surface recordings has been considered a distinctive sign of outer hair cell integrity in patients with auditory neuropathy. This report focuses on the results of an analysis performed on cochlear microphonic recorded by transtympanic electrocochleography in response to clicks in 502 subjects with normal hearing threshold or various degrees of hearing impairment, and in 20 patients with auditory neuropathy. Cochlear microphonics recorded in normally-hearing and hearing-impaired ears showed amplitudes decreasing by the elevation of compound action potential. Cochlear microphonic responses were clearly detected in ears with profound hearing loss. After separating recordings according to the presence or absence of central nervous system pathology (CNS+ and CNS-, respectively), cochlear microphonic amplitude was significantly higher in CNS+ than in CNS- subjects with normally-hearing ears and at 70 dB nHL compound action potential threshold. Cochlear microphonic responses were detected in all auditory neuropathy patients, with similar amplitudes and thresholds to those calculated for normally-hearing CNS- subjects. Cochlear microphonic duration was significantly higher in auditory neuropathy and normally-hearing CNS+ patients compared to CNS- subjects. Our results show that: 1. cochlear microphonic detection is not a distinctive feature of auditory neuropathy; 2. CNS+ subjects showed enhancement in cochlear microphonic amplitude and duration, possibly due to efferent system dysfunction; 3. long-lasting, high frequency cochlear microphonics with amplitudes comparable to those obtained from CNS- ears were found in auditory neuropathy patients. This could result from a variable combination of afferent compartment lesion, efferent system dysfacilitation and loss of outer hair cells.

Riassunto
Il microfonico cocleare (CM) è un potenziale recettoriale che si ritiene venga generato principalmente dalle cellule ciliate esterne (OHCs). Il suo rilevamento mediante registrazione di superficie è stato considerato un segnale distintivo dell’integrità delle OHCs nei pazienti affetti da neuropatia uditiva (AN). In questo lavoro vengono presentati i risultati relativi ad una analisi del CM registrato mediante elettrococleografia transtimpanica in risposta a click effettuata in 502 pazienti con soglia uditiva normale o con diversi gradi di deficit uditivo ed in 20 pazienti con AN. Il CM è stato riscontrato in tutti gli orecchi con soglia normale o con ipoacusia profonda. Suddividendo i soggetti esaminati in base alla presenza (CNS+) o all’assenza (CNS-) di patologia del Sistema Nervoso Centrale, si è notato che l’ampiezza del CM è significativamente maggiore nei soggetti CNS+ rispetto ai soggetti CNS- sia nei soggetti con un udito normale che in quelli con una soglia del potenziale d’azione a 70 dB nHL. Le risposte CM sono state state rilevate in tutti i pazienti con neuropatia uditiva con soglie e ampiezze simili a quelle presenti nei soggetti CNS- con udito normale. La durata del CM è risultata significativamente maggiore nei pazienti CNS+ rispetto ai pazienti CNS- con udito normale. I nostri risultati dimostrano che: 1. il rilevamento del CM non è una caratteristica peculiare della AN; 2. i soggetti CNS+ presentano un incremento di ampiezza e di durata del CM, probabilmente dovuto ad una disfacilitazione del sistema efferente; 3. CM ad alta frequenza, di lunga durata e di ampiezza comparabile a quella ottenuta negli orecchi dei pazienti CNS-, sono stati riscontrati nei pazienti con AN. Questo potrebbe essere dovuto alla combinazione variabile di fattori quali una lesione nel compartimento efferente, la disfacilitazione del sistema efferente e la perdita di OHCs.
Introduction

The cochlear microphonic (CM) is a gross potential generated by cochlear hair cells that can be recorded in humans and experimental animals at several recording sites. It is believed to result from the vector sum of the extra-cellular components of receptor potentials arising in inner (IHCs) and outer hair cells (OHCs), with the latter contributing more to CM generation on account of their greater number. On the basis of the estimated length constant of this extra-cellular activity, the CM recorded at the promontory or in the ear canal is held to arise primarily from the more basal portions of the cochlea, while the apical regions make a negligible contribution to its generation.

CM has been obtained in humans by recording intracellular or extra-cellular electrocochleography (ECochG). According to several reports, promontory recordings are considered to be more sensitive than ear canal recordings, and this is likely to result from the better signal-to-noise ratio of the promontory recordings. CM has also been obtained in surface recordings by means of skin electrodes and their low signal-to-noise ratio compared to both intratympanic and ear canal recordings are fully justified by the distance between the active electrode and the source of CM generation, since even higher response attenuation is expected than that between ear canal and promontory recordings.

CM has always been considered to have extremely limited clinical use, although much attention has been focused on developing analytical techniques to cancel it from electrocochleographic responses with the aim of extracting the compound action potential (CAP). Therefore, extensive data concerning CM parameters in normally-hearing ears and in ears with various degrees of threshold elevation are not yet available. Recently, however, CM recordings have attracted new interest following the identification of auditory neuropathy (AN), a disorder characterized by impairment of peripheral auditory function with preservation of OHC integrity. According to the most widely accepted view, this disorder almost always results from impaired function of auditory nerve fibres through demyelination and axonal loss, but, in some patients, it might also be caused by lesions involving terminal auditory nerve dendrites, IHCs and/or the synapses with auditory nerve fibres. AN patients, typically, present severe impairment of speech perception, which appears reduced out of proportion to the pure tone threshold, while auditory brainstem responses (ABRs) are absent or show severe abnormalities. These findings have been related to reduced temporal synchrony of auditory nerve activity resulting from all possible combinations of demyelination and axonal loss in auditory nerve fibres, IHC loss and synaptic disruption. It is generally accepted that preservation of OHC integrity, in AN, is indicated by the detection of otoacoustic emissions and/or cochlear microphonic, the latter usually being obtained by surface-recordings.

Since several studies have reported the disappearance of otoacoustic emissions or their absence in a large number of AN patients, assessment of OHC integrity and thus, more generally, the diagnosis of AN, rely solely on CM detection in surface recordings. However, the relationship between pure CM detection in surface recordings and normal OHC function remains to be fully elucidated. Firstly, CM is almost always detected when recording transtympanic ECochG in ears with varying degrees of hearing impairment or even profound hearing loss and thus, in the presence of extensive OHCs loss. Secondly, the fact that CM was undetectable in surface recordings in some patients with sensorineural hearing loss must be due to the lower signal-to-noise ratio involved in surface recordings compared to transtympanic recordings. Thus CM detection in surface recordings cannot be considered an irrevocable sign of normal hearing threshold and/or OHCs integrity. Moreover, there is still a lack of normative data on surface-recorded CMs in normally-hearing and hearing-impaired subjects, except for the report by Starr et al., focusing on CM amplitudes obtained in a group of normally-hearing subjects.

Since transtympanic electrocochleography is considered a “gold standard” technique yielding the strongest cochlear microphonic recordings in humans, it seems the most suitable tool when comparing CM responses recorded in AN patients with those obtained in normally-hearing ears or in ears with varying degrees of hearing impairment. However, most of the data available from transtympanic electrocochleography concern the neural response (compound action potential, CAP) and the summating potential (SP), due to their potential clinical use. Conversely, no studies specifically address the problem of CM parameters in normally-hearing ears and in ears presenting hearing loss or definite peripheral pathology, such as auditory neuropathy. The only paper focusing on CM recordings, obtained by transtympanic electrocochleography in humans, is by Aran and Charlet de Sauvage, who found that CM amplitudes decreased with the CAP threshold elevation in a large sample of ears. However, the subjects included in their study showed a wide age range, and it has been reported that age significantly affects the CM amplitudes calculated for the surface recordings obtained in normally-hearing ears.

When analysing CM responses, any effects that might be related to an efferent system dysfunction should be taken into account, since changes in efferent system...
activity are known to influence cochlear function through modifications of OHC electrical activity 28. The possibility that a brain dysfunction could underlie abnormal functioning of the efferent system has been stressed in several studies 29-32, in which it was reported that both Central Nervous System (CNS) lesions and behavioural disorders could induce abnormal functioning of the medial olivo-cochlear (MOC) system, as evaluated by suppression of transient evoked otoacoustic emissions (TEOAEs) in response to white noise presentation to the opposite ear. Aim of this study was to evaluate CM amplitudes and thresholds in normally-hearing ears and in ears with varying degrees of CAP threshold elevation, and compare them with corresponding values obtained from a group of patients affected by AN. Subjects were divided into two groups according to the presence of CNS disorder in order to identify ears with possible efferent system dysfunction which could influence auditory peripheral activity and, possibly, CM parameters. Results presented herein are drawn from an analysis performed on data from 522 subjects undergoing transtympanic ECochG for diagnostic purposes.

Methods

Subjects
This study population comprised 522 patients (310 male, 212 female) referred for transtympanic ECochG from October 1979 to January 2005. Mean age at the time of electrophysiological testing was 3.1 years ± 3.9 (range 7 months - 47 years). Only recordings obtained from ears with normal otoscopy and tympanometry were included (ears = 859, right 445, left 414). Twenty patients were affected by AN as they exhibited distortion product otoacoustic emissions (DPOAEs) and no ABRs. All non-AN patients (AN- subjects) were assigned to one of two groups according to the presence (CNS+, n = 187) or absence (CNS-, n = 315) of CNS pathology. Inclusion criteria for the CNS+ group was in accordance with the classification of Fischbein et al. 33 for CNS lesions revealed by neuro-radiological evaluation (brain computed tompography (CT) scans, magnetic resonance imaging (MRI)). In addition, patients with a positive neurological evaluation for motor and/or cognitive dysfunction and behavioural disorders were included in the CNS+ group (Table I).

ECochG recording procedure
Transtympanic ECochG was performed under general anaesthesia (nitrous oxide, sevoflurane) in children and with local anaesthesia (lidocaine, 10%) in adults. Patients were lying on a bed in an acoustically- and electrically-shielded room. The transtympanic electrode was a sterilised stainless steel needle which was insulated except for the tip; this was placed on the promontory wall with the aid of an operating microscope. Two silver-chloride cup electrodes applied to the earlobe and forehead served as reference and ground electrodes, respectively. Stimuli consisted of rarefaction and condensation 0.1 ms clicks, delivered separately in free-field by means of two high frequency drivers (RCF, N580, 8 Ω) mounted on a single polyurethane horn (RCF, H6040), with a maximum intensity of 120 dB pe SPL (corresponding to 90 dB nHL, referred to the psychoacoustical threshold of normally-hearing subjects). The stimulus was calibrated in free-field by means of a Bruel and Kjaer 4165 microphone (mounted on the 800 B Larson-Davis sound level meter), positioned 1 m from the base of the polyurethane horn, corresponding to the distance between the patient’s ear and the horn. Signals were differentially amplified (50000), filtered (5-8000 Hz) and sent to a computer for analogue-to-digital conversion (40 kHz, 20 kHz before 1990), displaying and averaging. The procedure of averaging the responses evoked separately by condensation and rarefaction clicks was applied to the electrocochleographic responses in order to extract the CAP. The resulting curve was then subtracted from the response evoked by condensation clicks to obtain the CM. The CAP threshold was assumed to be 130 dB pe SPL when no neural response was identified at the maximum stimulation level (120 dB pe SPL).

DPOAEs
Of 522 patients (309 ears), 166 underwent DPOAE recording under general anaesthesia before performing ECochG. DPOAEs were recorded by the Virtual 330 system in subjects admitted to ECochG from 1998 to 1999 (n = 42). f1 and f2 levels were kept at 65 and 55 dB SPL, respectively, while the f2/f1 ratio was 1.22. Primary tones were stepped, in regular in-
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retrvals, from 1 to 4 kHz (1/12 octave steps from 1 to 4 kHz). DPOAEs were measured as an average of 4 separate spectral averages and 8 time averages were performed. Each test lasted approximately 2 minutes. In subjects admitted to ECochG from 1999 to 2005 (n = 122), DPOAEs were obtained by means of the ILO-92 OAE system. The primary tones f1 and f2 were presented at 70 dB SPL and the f2/f1 ratio was kept at 1.21. The frequency was changed in 1/4 octave steps from 708 to 6299 Hz. Four spectral averages were calculated for each stimulus condition. Duration of the test was maintained at approximately 2 minutes.

DATA ANALYSIS

CM amplitude was measured as the difference between the maximum peak of a given polarity and the maximum peak of the opposite polarity. Differences in CM amplitudes calculated at the maximum stimulation intensity (120 dB peSPL) for the variables of group (presence or absence of CNS pathology), sex (male, female) and ear (right, left) were evaluated using variance analysis (SPSS 11.5). Regression procedures were used to examine the relationships between CM amplitude and CAP or CM threshold. Student t test for unrelated samples was used to evaluate differences in CM amplitude and threshold between AN patients and CNS- or CNS+ groups with normal CAP threshold. A p value of < 0.05 was considered significant.

Magnitude and phase spectra of the average-response waveforms were calculated by Fourier transformation (Labview 7.1, National Instruments). Analytic signal representation was used to estimate the instantaneous frequency (IF) of averaged curves (Labview 7.1, National Instruments).

Results

The aetiology of hearing impairment in the AN- subjects is reported in Table II together with the percentage of CNS+ patients. It can be seen that no definite diagnosis could be made in more than one third of the subjects. The proportion of CNS+ patients was relatively low in this group and was represented mainly by cognitive and behavioural disorders or neurological diseases. When considering the group with neonatal risk factors for hearing impairment, almost 50% of the patients showed CNS involvement. The proportion of CNS+ patients was even higher in subjects with prenatal or acquired risk factors for hearing impairment, the latter group being almost completely represented by meningitis. Most CNS+ subjects in the hereditary group showed an associated syndrome.

Electrocochleographic recordings obtained from three representative ears with normal threshold, elevated CAP threshold or absence of neural response at the maximum stimulation intensity (120 dB peSPL) are reported in Figure 1. CM was clearly identifiable in the ear lacking CAP, in spite of profound deafness. Both CM amplitude and threshold appeared to depend upon the CAP threshold, since the highest amplitude and lowest threshold were found in normally-hearing ears. In the presence of sensorineural hearing loss, amplitude attenuation and CM threshold elevation increased with the degree of hearing impairment.

EARS WITH NORMAL CAP THRESHOLD, CNS- AND CNS+ SUBJECTS

Hearing threshold was assumed to be normal when CAP was detected at an intensity of 60 dB peSPL (corresponding to 30 dB nHL) or lower. According to

<table>
<thead>
<tr>
<th>Aetiology of hearing loss</th>
<th>Subjects</th>
<th>CNS+ Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unknown origin</strong></td>
<td>34.2</td>
<td>12.5</td>
</tr>
<tr>
<td><strong>Hereditary</strong></td>
<td>24.1</td>
<td>8.3</td>
</tr>
<tr>
<td>(familiarity, genetic syndromic, genetic non-syndromic)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Prenatal</strong></td>
<td>10.5</td>
<td>6.4</td>
</tr>
<tr>
<td>(Toxoplasmosis, Rubella, CMV, Herpes Simplex, drugs, Rh incompatibility, other infections)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Neonatal</strong></td>
<td>25.1</td>
<td>12.3</td>
</tr>
<tr>
<td>(prematurity, low birth weight, low Apgar score, mechanical ventilation, hyperbilirubinaemia, ototoxic medications, dystocia)</td>
<td></td>
<td></td>
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<tr>
<td><strong>Acquired</strong></td>
<td>6.1</td>
<td>3.6</td>
</tr>
<tr>
<td>(bacterial meningitis, neurological diseases, drugs)</td>
<td></td>
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</table>
this criterion, our sample included 139 ears (95 sub-
jects, mean age 3.1 ± 1.9 years, range 8 months - 10
years).

Mean CAP threshold was 41.1 ± 9.5 dB peSPL while
mean CM threshold and mean CM amplitude, calcu-
lated at the maximum stimulation intensity (120 dB
peSPL), were 80.9 ± 10.2 dB peSPL and 29.1 ± 33.1
µV, respectively.

No significant effect of sex, ear or age was found ei-
ther on the CM threshold or amplitude. Conversely,
the variable (presence or absence of CNS pathology)
of group significantly affected CM amplitude (ANO-
VA, p < 0.05), which was higher in the CNS+ (107
ears, CM amplitude at 120 dB peSPL 32.2 ± 36.6
µV) than in the CNS- group (32 ears, CM amplitude
at 120 dB peSPL 18.8 ± 12.8 µV) (Student t test for
unrelated samples, p < 0.05). The presence of CNS
disorders was not found to have any significant effect
on CM threshold.

The effect of CNS pathology on CM amplitude is il-
ustrated in Figure 2 for the electrocochleographic
responses recorded from two representative subjects
with normal CAP threshold: one belonged to the
CNS- group (left side), the other was an autistic child
(right side). Note the high amplitude, low threshold
and “long-ringing” appearance of the CM recorded
in the CNS+ patient.

**EARS WITH NO CAP RESPONSE**

No neural response could be detected in 202 ears
(107 subjects, mean age 2.6 ± 4.2 years, range 8
months - 29 years) at the maximum stimulation in-
tensity (120 dB peSPL). These ears were presumed to
have profound hearing loss and thus an arbitrary val-
ue of 130 dB peSPL was assigned to the CAP thresh-
old.

CM was detected in all ears in this group in spite of
profound hearing loss. Mean CM threshold and am-
plitude obtained at the maximum stimulation intensi-
ty (120 dB peSPL) were 99.1 ± 7.9 dB peSPL and 7.5
± 9.7 µV, respectively. No significant effect of sex,

ear, age or group (CNS-/CNS+ subjects) was found
either on CM threshold or amplitude.

CM amplitude was smaller and the CM threshold

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**Fig. 1.** Electrocochleography recordings obtained from three representative ears showing normal CAP threshold, ele-
vated CAP threshold and the absence of neural response at maximum stimulation intensity (120 dB pe SPL). In this and
following figures, CAP and CM traces, obtained by classic procedure of averaging recordings to condensation and rar-
efaction clicks, are reported for decreasing stimulation intensities.
lower than normally-hearing or elevated CAP threshold ears. Nevertheless, mean CM amplitude calculated at the maximum stimulation intensity (120 dB peSPL) was significantly higher than that obtained from ears with a 120 dB peSPL CAP threshold (Student t test for unrelated samples, p < 0.05).

EARS WITH ELEVATED CAP THRESHOLDS
A total of 478 ears (300 subjects, mean age 3.1 ± 1.1 years, range 7 months - 28 years) presented a CAP with an elevated threshold compared to normally-hearing subjects. The ears included in this group showed variable levels of CAP threshold elevation compared to normally-hearing ears and thus variable degrees of estimated sensorineural hearing loss. All ears were divided into 10 dB classes according to CAP threshold values.

Sex of the patient and ear side had no effect either on CM threshold or amplitude. No age or group effects were found, except for ears with the 100 dB CAP threshold. In this class, the presence of a CNS disorder significantly affected both CM threshold (ANOVA, p < 0.05) and amplitude (ANOVA, p < 0.05, CM amplitude calculated at 120 dB peSPL). Amplitudes were higher (Student t test for unrelated samples, p < 0.05) and thresholds were lower (Student t test for unrelated samples, p < 0.05) in patients in the CNS+ group (43 ears) compared to those in the CNS- group (71 ears). Moreover, ears with a 100 dB CAP threshold showed a significant age effect both on CM threshold (ANOVA, p < 0.01) and amplitude (ANOVA, p < 0.0001, CM amplitude calculated at 120 dB peSPL), with the latter increasing as a function of age up to 5 years.
RELATIONSHIP BETWEEN CAP THRESHOLD, CM THRESHOLD AND CM AMPLITUDE

Only AN- subjects (819 ears) were included in the analysis. Ears were divided into 10 dB classes on the basis of their CAP threshold. Individual CM amplitudes, calculated at 120 dB peSPL together with the corresponding mean values, are reported as a function of CM thresholds on the left side of Figure 3. CM amplitude and threshold proved to be related to each other (ANOVA, p < 0.0001), so that high CM amplitudes corresponded to low CM thresholds. The curve that best fitted individual values was an exponential curve (p < 0.0001, R = 0.70, y = 3327.65* e^{-0.067476*x}).

Both CM threshold and amplitude were related to CAP threshold (ANOVA, p < 0.0001). The right side of Figure 3 shows the individual CM amplitudes together with the corresponding mean values as a function of CAP thresholds. The highest mean amplitude of CM was observed in ears with normal or slightly elevated CAP thresholds. The curve that best fitted individual CAP amplitudes values was an exponential curve (p < 0.0001, R = 0.48, y = 38.90* e^{-0.018876*x}). CM thresholds were linearly related to CAP thresholds (p < 0.0001, R = 0.62, y = 0.55*x + 0.40).

DPOAE RECORDING

Otoacoustic emissions were recorded in 166 subjects. DPOAEs were found in 72 out of 309 ears (39 out of 166 subjects), when AN patients were included in the group. Figure 4 shows the percentage presence of DPOAEs at various CAP thresholds (open squares, interrupted line). The absolute number of all ears showing DPOAEs is indicated for each CAP threshold value. It can be seen that DPOAEs were found in almost all ears with a normal CAP threshold (≤ 60 dB peSPL), while percentage occurrence dropped to 42.9% at the 70 dB CAP threshold, and to 21.1% at the 100 dB CAP threshold. The occurrence of DPOAEs substantially decreased when AN patients were excluded from the calculation (Fig. 4, closed squares). In particular, there was a drop in the occurrence of DPOAEs in the 70-90 dB CAP threshold range, while the five ears with profound deafness (130 dB CAP threshold) were no longer included in the group.

AUDITORY NEUROPATHY

Clinical and audiological features found in AN patients are outlined in Table III. Of these patients, 50% were children presenting one or more risk factors for hearing loss related to neonatal illnesses. However, no risk factors for hearing loss could be detected in the clinical history of 3 children, while 3 other patients (2 adults and one child) presented optic atrophy. Of the 20 AN subjects, 6 (5 adults, one 7-year-old child) also presented marked impairment of speech perception, which appeared to be reduced out of proportion to the pure tone threshold.

CM curves obtained in individual ears at the maximum stimulation intensity (120 dB peSPL) are shown in Figure 5. Both CM amplitude and duration showed a high degree of variability. Relevant changes in oscillation frequencies across ears were also observed.
Mean CM threshold and amplitude calculated at 120 dB peSPL of stimulation intensity were 88.0 ± 11.4 dB peSPL and 13.5 ± 26.8 µV, respectively. According to the definition of AN, the OHC compartment must function properly in these patients and thus the CM amplitude and threshold could be compared to the corresponding values obtained from normally-hearing ears (CNS-, CNS+). The graph in the upper part of Figure 6 summarizes the differences, between the CNS-, CNS+ and AN groups.

The mean CAP threshold was significantly higher in AN patients (89.7 ± 22.0 dB peSPL) than in both the CNS- and CNS+ groups (Student t test for unrelated samples, p < 0.0001). No significant differences in CM threshold were found between CNS- and AN subjects, while the mean CM threshold calculated for CNS+ patients was significantly lower than that obtained for the AN group (Student t test for unrelated samples, p < 0.001).

No significant differences in CM amplitude were observed between AN and CNS- subjects, while amplitude values proved to be higher in CNS+ subjects than in both CNS- (see above, p < 0.05, Student t test for unrelated samples) and AN patients (p < 0.01, Student t test for unrelated samples). In addition, the CM amplitude obtained in AN subjects did not differ significantly from that calculated for the 70, 80, 90 and 100 dB CAP thresholds in the CNS- group. Regarding large CM responses detected in some AN patients, 3 out of 40 AN ears (7.5%) (3 subjects) had an abnormally elevated amplitude compared to normally-hearing CNS- subjects (> 1 SD above the mean value), while high amplitude CMs were found in 35 out of 107 ears (32.7%) (31 patients) in normally-hearing CNS+ subjects.

The grand average (GA) of cochlear microphonic responses obtained from CNS- (17 ears), CNS+ (27 ears) and AN subjects (40 ears) was calculated for illustrative purposes. The resulting curves are shown in the lower part of Figure 6. Differences in amplitude between groups fit quite well with the results of the statistical evaluation.

**CM Duration**

Since CM has been described as “long-ringing” in some patients with AN, CM duration was calculated and compared to that of normally-hearing subjects (CNS-, CNS+).

When considering electrical activity following the first portion of the cochlear microphonic at the maximum stimulation intensity (120 dB peSPL), almost all subjects showed a reversion in phase between rarefaction and condensation stimuli. This is shown for two representative CNS- subjects in Figure 7. This

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**Fig. 4.** Percentage of DPOAE presence at various CAP thresholds. Open squares: all ears; closed squares: percent occurrence of DPOAE obtained excluding AN ears. N and n: total number of ears exhibiting DPOAEs with or without the inclusion of AN patients, respectively.
finding clearly indicates that the low-amplitude oscillations following the first CM portion represent the enduring CM (see also Figs. 1, 2). This prevented us from identifying the CM end by visual inspection of individual curves. Thus, in order to evaluate the duration CM, both the instantaneous frequency (IF) and FFT spectrum were calculated on the CMs recorded at 120 dB peSPL in CNS-, CNS+ and AN subjects. The CM curves together with the corresponding instantaneous frequency graphs and FFT spectra obtained in four representative subjects are shown in Figure 8. The rapid increase in IF corresponds to CM onset. It can be seen that the IF increase is maintained for about 2 ms in the CM obtained from the CNS- subject, while it lasts throughout the entire recording segment following CM onset in CNS+ and AN ears. Mean CM duration was 4.36 ± 1.97 ms, 7.99 ± 2.49 ms and 6.77 ± 2.58 ms for CNS-, CNS+ and AN ears, respectively. Significant differences in CM duration were found between CNS- and CNS+ (p < 0.001, Student t test for unrelated samples) and AN groups (p < 0.01) were found, while CM duration did not differ significantly between CNS+ and AN subjects. On account of the oscillations in IF over time, the main CM oscillation was also evaluated by calculating the FFT over the whole CM curve as well as on the time window corresponding to the response segment in which a stable increase in IF was found compared to the time preceding CM onset. Mean frequency obtained for CNS-, CNS+ and AN ears was 2320 ± 395 Hz (n = 12), 2330 ± 358 Hz (n = 20) and 2922 ± 875 Hz (n = 27), respectively. The mean oscillation frequency calculated for AN subjects proved significantly higher than that obtained both for CNS- (p < 0.05, Student t test for unrelated samples) and CNS+ ears (p < 0.01), while no significant differences were found between the CNS- and CNS+ groups. Mean frequencies higher than 2600 Hz (mean value calculated for normally-hearing ears plus one standard deviation) were found in 15 out of 27 AN ears. An example is reported in the third line.
of Figure 8. Both the IF graph and FFT spectrum, calculated over the whole time window, show that the main CM frequency is consistently higher in this curve compared to the remaining traces the frequency of which is centred on about 2500 Hz.

**Discussion**

We recorded transtympanic electrocochleography as a second-level procedure to estimate hearing threshold in non-cooperative subjects when ABR showed a low degree of reliability and/or all non-invasive procedures failed to yield unequivocal results.

ECochG results from superimposition of two receptor potentials – the cochlear microphonic and the summating potential – and the neural response (CAP). The CM obtained from promontory recordings is acknowledged as a robust potential originating mainly from the activation of hair cells located in the more basal regions of the cochlea, with the OHCs contributing primarily to the generation of response. Sensitivity is progressively lowered when the active electrode is moved away from the promontory, towards the ear canal or the mastoid, thus that recordings obtained by surface electrodes are likely to show the highest degree of response attenuation. Accordingly, the reported CM amplitudes calculated for surface recordings in normally-hearing subjects appear to be much smaller than those reported in our study or in the paper by Aran and Charlet de Sauvage. It is, therefore, not surprising that CM was not obtained by surface recordings in some subjects presenting hearing impairment of varying degrees as the response amplitude greatly decreases as the CAP threshold increases.

**Normative Data in Normally-Hearing and Hearing-Impaired Ears**

Our data on CM amplitude closely agree with the results reported by Aran and Charlet de Sauvage, who recorded transtympanic ECochG in normally-hearing ears and in ears with mild to profound hearing impairment. The trend reported by these Authors, whereby mean CM amplitude decreases as a function of CAP threshold, appears to be very similar to our
findings, except for the absolute CM amplitudes, which are lower than our values. We have no definite explanation for this discrepancy. One hypothesis is that our sample also included patients with CNS pathology, who exhibited significantly higher CM amplitude than subjects with no signs of CNS involvement. However, when considering only CNS-ears, substantial differences in CM amplitudes remained. Another possible explanation concerns the differences in stimulation parameters, since small differences in click intensity, rise time and/or spectral content could lead to different hair cell recruiting patterns with, consequently, differences in CM amplitude. It is also worth mentioning that over 90% of our sample comprised children aged < 5 years, while a substantial number of adult patients must have been included in the study by Aran and Charlet de Sauvage. Since Starr et al. obtained a significantly negative correlation between age and amplitude of surface-recorded CMs in normal subjects, differences in amplitude between our data and those reported by Aran and Charlet de Sauvage might also be related to age differences between the two samples of subjects. Substantially lower CM amplitudes compared to our data were obtained by Schoonhoven et al. who performed transtympanic ECochG in children. However, unlike in our study, they used tonebursts at various octave frequencies with fixed rise time, so that differences in hair cell recruitment are to be expected.

When considering the results reported in the above-mentioned paper by Starr et al., we failed to find any decrease in CM amplitude with age in normally-hearing ears. We have no definite explanation for this finding. However, as mentioned above, our sample consisted almost entirely of young children, while the normal subjects enrolled by Starr et al. showed a wide age range. Conversely, we found analogies with the data reported in other studies concerning the effects of age on otoacoustic emissions recorded in childhood, since no changes in TEOAE amplitude have been observed from 1 to 5 years of age. Nevertheless, we are unable to explain the increase in amplitude with age found in the 100 dB CAP threshold group. This effect could, to a certain extent, reflect differences in age between CNS- and CNS+ subjects, with the CNS+ group being of a significantly higher mean age (p < 0.0001) than the CNS- group. This is because the age at audiological diagnosis is often higher in children with associated disabilities than in those who present only hearing impairment.

Cochlear microphonics were identified in ears lacking CAP at the maximum stimulation intensity, in agreement with other reports. CM amplitude proved to be less than half the value calculated for normally-hearing CNS- subjects, and this is likely to reflect the considerable hair cell loss found in severe cochlear lesions. Given the wide spectrum of aetiologies in our sample, it is not possible to predict the type and number of residual hair cells contributing to CM generation in ears with profound hearing loss. Nevertheless, it does not seem unreasonable to assume that the residual hair cells are made up almost entirely of IHCs. According to Dallos, the price to be paid for the high level of OHC specialization, in supplying both the high sensitivity and frequency tuning of the auditory system, increases OHC vulnerability to damage. Studies performed in animals indicate that OHCs are more vulnerable than IHCs to the effects of noise as well as the vast majority of ototoxic drugs. These concepts cannot easily be applied to congenital hearing loss in humans, due to the above-mentioned variability in aetiologies and the few histological studies available on post-mortem temporal bones in children. For instance, hereditary hearing impairments caused by different genetic mutations may show different histological patterns of hair cell loss. To our knowledge, the only extensive study performed in children was carried out in newborns who died in neonatal intensive care units. The majority of infants presumed to have an elevated hearing threshold, as evaluated by ABR recording,
presented OHCs loss or alteration, associated or not associated with the IHC lesion. Moreover, many alterations in auditory perception, observed in hearing-impaired subjects, can be accounted for by the absence of a cochlear amplifier mechanism like that provided by OHCs in healthy cochleas. Therefore, it does not seem unreasonable to assume that residual IHCs contribute almost entirely to CM generation in our subjects with profound deafness. This is not in contrast with the findings reported in some studies performed in chinchillas, which have shown that IHC loss has only a minor effect on CM amplitude given the widely acknowledged IHC contribution to CM generation. Overall, it seems reasonable to assume that the reduction in CM amplitude observed at low CAP thresholds is strictly related to OHC loss, while at very high CAP thresholds, the CM amplitude may critically depend upon the amount of residual IHCs.

Surprisingly, the CM amplitude calculated for ears with no neural response at maximum stimulation intensity was significantly higher than that obtained at the 120 dB CAP threshold. The same observation was reported by Aran and Charlet de Sauvage. We have no definite explanation for this finding. The presence of the neural response, at the 120 dB CAP threshold, may, in some way, influence CM extraction by the classical procedure of averaging the curves recorded in response to clicks of opposite polarity. This hypothesis is feasible considering that pathological cochleas could give rise to asymmetrical neural or receptor responses when utilizing stimuli of opposite polarity. On the other hand, extensive hair cell loss may, in some way, increase the length constant of individual extra-cellular potentials arising along the cochlear partition through a reduction in the electrical impedance of tissues interposed between the residual hair cells and the recording electrode.

The highly significant correlation between CM thresholds and amplitudes appears straightforward, since CM thresholds are critically dependent upon the number of averaged sweeps at a given stimulus intensity. If the number of averages is fixed, the
threshold will be related to the observed CM amplitude at a given stimulus intensity, namely at 120 dB peSPL. The linear relationship between CAP and CM thresholds could indicate that the same phenomenon underlies the variations in these two variables, namely OHC loss.

CNS pathology

Patients included in the CNS+ group showed a wide spectrum of CNS pathologies (Table II). When considering ECochG recordings from 1970 to 1990, only a few subjects had undergone evaluation by CT or MRI. Thereafter, about 80% of the tested subjects had positive radiological evaluation, showing lesions involving cortical and/or subcortical neural structures, which appeared to be widespread in the vast majority of cases. About 15% of CNS+ patients presented behavioural disorders without evidence of neurological involvement. These consisted essentially of autism or autistic-like syndromes. The CM amplitudes calculated in normally-hearing ears were significantly higher in CNS+ patients than in subjects without CNS pathology; this also applied to the 100 dB CAP threshold group, in which significant differences in CM thresholds between CNS+ and CNS- subjects were also found.

Several studies have demonstrated that pathological processes involving the brain can induce a dysfunction of the medial olivo-cochlear (MOC) system, evaluated by suppression of TEOAEs recorded in one ear in response to white noise presentation to the contralateral ear. Lesions involving cerebral hemispheres as well as midline petrous apex lesions have been found to be associated with abnormal efferent system functioning. Moreover, CNS dysfunctions, such as auditory processing disorders and autism, exhibit decreased activity of the MOC system, in the former, and an asymmetrical pattern of activation between the two ears, in the latter.

It is, therefore, reasonable to hypothesize that the CM enhancement observed in CNS+ patients could be related to a dysfunction of the efferent system and/or cortical centres modulating its activity. This hypothesis seems very probable in patients with behavioural disorders, a large number of which were present in our sample. The remaining subjects showed widespread lesions involving both cortical and subcortical structures, thereby precluding a precise classification based on the site of the lesion. Nevertheless, considering the wide extension of the efferent system throughout the brainstem and its multiple interrelations with the auditory cortex, lesions localized in several CNS structures could conceivably induce efferent system dysfunction through abnormal functioning of the MOC system itself or a reduction in the modulation activity provided by cortical centres.

It is widely accepted that the ultimate effect of medial efferent system activation on the auditory periphery consists of a reduction in auditory nerve fibre activity due to a decreased gain in cochlear amplification provided by the OHCs. It can, therefore, be hypothesized that the enhancement of CM amplitude detected in CNS+ patients could stem from a reduction in MOC system activity, which, in turn, results in a decreased inhibitory effect on OHCs and thus in an enhancement of cochlear amplification.

Differences in amplitude between the CNS+ and CNS- groups were not found in the hearing-impaired subjects, except in the 100 dB CAP threshold group. This is probably due to the high variability both in OHC and IHC loss distribution throughout the cochlear partition in hearing impairment of different aetiologies. Hence, a given CAP threshold does not necessarily involve the same amount and distribution of OHC loss in the basal portion of the cochlea, where CM is believed to be generated. Nevertheless, we are unable to explain the differences observed, in CM amplitude and threshold, between CNS+ and CNS- subjects at the 100 dB peSPL CAP threshold. Since it has been reported that CAP threshold elevation, measured in rats after noise exposure, depends on the amount of OHC loss in the more basal portions of the cochlea, it is conceivable that the 100 dB threshold could, in some way, correspond to the point of maximum OHC loss in this cochlear region, which could make the ears in this group more uniform in terms of hair-cell contribution to CM generation.

It is worthwhile stressing that the distinction between CNS+ and CNS- groups was made on the basis of a diagnosed disability related to a CNS dysfunction or lesion. Using this criterion, subjects were divided into two groups, which showed differences in CM amplitude, at two different CAP thresholds. As far as this result is concerned, we hypothesized that differences in CM amplitude could be accounted for by admitting the existence of an efferent system dysfunction in CNS+ patients. Obviously, this does not mean that all subjects included in the CNS+ group had an alteration of the MOC system, rather it suggests that the existence of abnormal efferent system functioning in a given number of patients of this group could bias CM amplitudes towards higher values compared to those obtained in subjects without signs of CNS involvement. From this viewpoint, it is conceivable that the CNS+ group includes patients presenting alterations in the cortical control of the efferent system, disorders of the brainstem affecting the efferent system itself or, possibly, no efferent system dysfunction.

AUDITORY NEUROPATHY AND DPOAEs

It is generally accepted that the detection of otoacoustic emissions and/or CMs indicates preservation of OHC
Table III. Clinical and audiological findings in auditory neuropathy. Age, sex, aetiology and clinical findings (left). Right: indications on CM amplitude (large > 1 SD above mean CNS- value, small < 1 SD below mean CNS- value), CAP thresholds and pure tone thresholds (PTA). FF refers to warble tones presented in free-field. Asterisks: ears lacking DPOAEs.

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integrity in patients with AN. The AN subjects included in our study displayed DPOAEs bilaterally (16 out of 19 subjects) or at least in one ear. All had strong CMs which were detectable in electrocochleography recordings (Fig. 7), and CM amplitude was not significantly different from that in CNS-subjects with normal hearing or a slightly elevated CAP threshold.

These findings suggest that the presence of CM in transtympanic recordings is not a distinctive sign of OHCs integrity, since it was found in ears with variable degrees of hearing impairment, ranging from
mild to profound. Moreover, CM amplitudes varied considerably within a given CAP threshold group; therefore, CM responses with the same magnitude could correspond to different CAP thresholds and thus to different OHC loss. Furthermore, as reported above, strong CM responses were recorded in ears with profound hearing loss, although the vast majority of OHCs are believed to have been lost. From this viewpoint, detection of CMs in surface recordings cannot be considered an invariable sign of OHCs integrity, since it largely depends on the likelihood of CM being picked up by surface electrodes. For example, a high amplitude CM corresponding to a considerable degree of OHC loss is likely to be recorded by skin electrodes, but obviously this finding does not indicate preservation of OHC integrity. This scenario becomes even more complicated if we hypothesize the concomitant association of an afferent system dysfunction related to CNS pathology. Overall, while pure detection of CMs in surface recordings seems to be unlikely in profound or severe deafness because of the low CM amplitude, it cannot be ruled out a priori in the presence of considerable OHCs loss. This means that, while the presence of surface-recorded CM in patients who already have a diagnosis of AN could be of value, the reverse is not true, i.e., when most other signs of auditory neuropathy are absent, the detection of CM in surface recordings is not significant at all.

In the comparison between CM amplitudes obtained in CNS- and AN subjects in transtympanic recordings, amplitude values calculated for AN patients were not significantly different from those obtained from normally-hearing ears in the absence of CNS pathology. Interestingly, however, no significant differences in CM amplitude were found between AN subjects and CNS- patients showing some degree of hearing impairment with CAP thresholds equal or lower than 100 dB peSPL. Bearing in mind that the percentage of OHC loss is strictly dependent on the CAP threshold elevation, the CM amplitude obtained in AN subjects reflects the contribution of OHCs activation or, at least, of a certain amount of them. In agreement with studies performed in man and experimental animals, we recorded DPOAEs in almost all subjects without AN with a CAP threshold within 30 dB nHL (60 dB peSPL). Only a few ears exhibiting CAP thresholds above this value had detectable DPOAEs, while no emissions were found in ears with CAP thresholds higher than 50 dB nHL. When including patients with AN, the DPOAE occurrence rate increased substantially in the 40-70 dB nHL CAP threshold range, and became positive at the 100 dB nHL CAP threshold due to DPOAEs detection in 5 ears with profound deafness.

Since the presence of DPOAEs, in our AN patients, did not seem to be related to the degree of hearing loss, CAP threshold elevation, in these subjects, must be primarily attributed to impairment of the cochlear afferent compartment rather than to OHC loss, as in the case of classical cochlear-related hearing impairment. This suggests that only detection of otoacoustic emission permits assessment of OHC integrity with a sufficient degree of reliability, whereas the cochlear microphonic is of limited use when performing transtympanic recordings and of no value in surface recordings. Nevertheless, detection of otoacoustic emission does not always indicate survival of the OHC compartment as a whole, since DPOAEs have been found in the presence of an OHCs loss in 40% of the total number of OHCs. Moreover, several studies have reported the disappearance of otoacoustic emissions or their absence in a large number of AN patients. These data would appear to be in agreement with the finding that transtympanic recordings performed in AN patients and CNS- subjects, with some degree of CAP threshold elevation, yield comparable CM amplitudes. Conceivably, therefore, AN ears could, at first, show subtle cochlear lesions which then develop into massive OHCs loss, which, in turn, leads to the disappearance of otoacoustic emissions. From this viewpoint, the lack of significant differences in CM amplitude between AN and hearing-impaired ears with a slightly elevated threshold acquires great value, although it should be considered with caution because of the presence of five adults in the our AN sample.

CM duration

Prolonged CM duration, in man, has been described by Gibbin et al. in normally-hearing and hearing-impaired subjects when performing transtympanic ECochG and the highest occurrence of long-ringing CMs was detected in normally-hearing ears. Moreover, several studies have reported CMs showing high amplitude and long-ringing appearance in some patients with AN. At first glance, prolonged CM duration was also found in many ECochG recordings, in the present study. Surprisingly, close examination of the ECochG traces obtained in response to rarefaction or condensation clicks revealed that a low-amplitude oscillation, lasting as long as 10 ms, followed the first high amplitude portion of the CM and reversed in phase in response to stimuli of opposite polarity. This phase inversion probably means that this low amplitude activity is the CM itself, lasting longer than previously believed. On the basis of this finding, evaluation of CM duration by visual inspection, represents a challenging if not impossible task. The application of analytical procedures aimed at obtaining the instantaneous frequency through Hilbert transformation associated with the Fourier analysis allowed us to calculate the main frequency of oscillation of CM
and its duration. However, caution is necessary in the use of this procedure for two reasons: i. CM morphology may prevent IF from remaining stable over time; ii. the classic procedure of averaging responses evoked by clicks of opposite polarity does not guarantee against CAP contamination of CM curves, and this is another threat against evaluating CM duration through the instantaneous frequency calculation. For these reasons, the CM curves submitted to analysis were carefully selected.

Evaluation of CM duration showed that long-lasting CMs were obtained in CNS+ and AN ears compared to CNS- subjects. In our opinion, this finding could be related, in some way, to cochlear amplifier enhancement induced by a decrease in efferent system activity, leading, in turn, to a certain degree of negative damping of the basilar membrane motion. Enhancement of OHC activity, due to reduced inhibition exerted by the efferent system has been hypothesized\(^6\) in order to explain the CM ringing observed in some AN patients. This hypothesis is also supported by the reduced or absent suppression of transient otocoustic emissions observed in response to the presentation of white noise to the contralateral ears in patients with auditory neuropathy\(^9\). From this viewpoint, CM amplitude enhancement acquires greater value than previously believed, despite being found in only a small percentage of AN patients.

It is more difficult to justify the differences in CM oscillation frequency between CNS- and CNS+ groups and AN patients. The simplest explanation could be related to differences in the audiometric threshold profile between patients, in that a relative hearing impairment at low frequencies, together with relative preservation of high frequencies, could lead to an even more consistent contribution to CM generation of the basal portions of the cochlea compared to ears with a flat audiometric profile. Unfortunately, no relationship was found, in our sample, between audiometric configuration and oscillation frequency, although this finding should be considered with caution due to the small number of ears involved. Alternatively, it might be speculated that changes in oscillation frequency could, in some way, result from changes in efferent system activity, exerting different effects at different sites in the cochlear partition.

**Conclusions**

The present report focuses on the analysis of cochlear microphonics obtained by transtympanic ECochG in a large sample of normally-hearing and hearing-impaired children. Results show that, in all patients, both CM amplitude and threshold are critically dependent upon the CAP threshold. However, CM is not abolished either at high CAP thresholds or in the absence of neural response at the maximum stimulation intensity. This finding challenges the widely accepted view that the CM is strictly related to OHC electrical activity with only a minor contribution from IHCs.

Comparison between DPOAE occurrence and CM amplitude distribution, at various CAP thresholds in AN- subjects, indicates that the presence of DPOAEs is a more sensitive index of hearing threshold preservation than CM amplitude.

The presence of a CNS pathology seems to enhance CM amplitude. This effect likely results from dysfunction of the medial efferent system through a reduced inhibitory influence on OHCs, leading, in turn, enhanced cochlear amplification. The reduction in efferent system activity could also be the mechanism responsible for the underlying long-lasting activity observed in many AN patients, who also showed enhancement of CM amplitude in some cases. The well-known disappearance of DPOAEs over time suggests that changes in CM amplitude and duration, in AN patients, result from a combination of OHC loss and efferent system dysfacilitation.

**References**


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