ORIGINAL ARTICLE

Cochlear Microphonic Latency

Julio Sanjuán Juaristi, a Mar Sanjuán Martínez-Conde

Unidad de Neurofisiología Experimental, Hospital Ramón y Cajal, Madrid, Spain

Received 26 September 2013; accepted 12 January 2014

KEYWORDS
Cochlear microphonic potentials; Response latency; Outer auditory hair cell; Mechanosensory transduction

Abstract
Introduction and objective: By using appropriate instrumentation, we have found that cochlear microphonics (CM) advance or delay their appearance, depending on the sound pressure that generates them. This time variation is on the order of microseconds. We have not found any reference to this behaviour, which is why we make the finding known.
Materials and method: We used the standard instrumentation specified for the study of CM. The method was based on the phase shift function of the CM according to the intensity of the stimulus.
Results: Latency was observed in CM, and we determined that latency time diminishes as the intensity of the stimulus increases.
Conclusions: From the sound stimulus to the bioelectric potential transduction, there is a time period of microseconds, the shorter the more powerful the stimulus. This suggests that electromechanical transduction is not a simple mechanical process.
© 2013 Elsevier España, S.L.U. All rights reserved.

PALABRAS CLAVE
Potenciales microfónicos cocleares; Tiempo de latencia; Células ciliadas externas; Transducción mecanosensitiva

Latencia de los microfónicos cocleares

Resumen
Introducción y objetivo: Empleando la instrumentación adecuada, venimos constatando que los microfónicos cocleares (MC) evocados adelantan o retrasan su aparición en función de la presión sonora que los genera. Esta variación en el tiempo es del orden de microsegundos. No hemos encontrado referencia alguna de este comportamiento, razón por la cual damos conocer el hallazgo.
Material y método: Se emplea instrumentación específica para el estudio de los MC. El método se basa en el desplazamiento de fase de los MC en función de la intensidad del estímulo.
Resultados: Se constata la latencia en los MC y se observa que la misma disminuye a medida que se incrementa la intensidad del estímulo.

Please cite this article as: Sanjuán Juaristi J, Sanjuán Martínez-Conde M. Latencia de los microfónicos cocleares. Acta Otorrinolaringol Esp. 2014; 65:231–236.

* Corresponding author.
E-mail address: marsanjuan@gmail.com (J. Sanjuán Juaristi).

2173-5735 © 2013 Elsevier España, S.L.U. All rights reserved.
Introduction

It takes approximately 300 ms for acoustic information to reach the cortex after stimulation of the organ of Corti.

It is considered that this time is the total latency of auditory perception. The potentials evoked in the relevant area can be situated according to the section studied.

In accordance with this latency Davis’ classifies the results as:

a. Cochlear microphonics (CM): representing electrical cochlear activity, with 0 latency.
b. Electro-cochleography: 1–4 ms. 2-5
c. Auditory evoked potentials of the brain stem: 2–12 ms.
d. Stable state potentials: 2–12 ms.
e. Medium latency potentials: 15–50 ms.
f. Long latency potentials: 50–300 ms.

Davis concludes that: “outer auditory hair cells lack latency as transducers of sound pressure in bioelectric potential”’. In this work we reject this hypothesis.

Electro-cochleography studies the microphonic response overall, not selectively as in our procedure. Wave I reflects the depolarisation of the auditory nerve evoked by click stimulation, stimulation of complex harmonic composition and consequently devoid of tonotopic selectivity.

Fig. 1 corresponds to the first CM trace in humans obtained bloodlessly, with contact electrodes alone, in 1985 (Hospital Ramón y Cajal). The discontinuous trace is due to the use of a plotter of the period. It can be observed how the CM response advances for stimuli 60, 70 and 80 dB. The response advancement can be seen for each increase in Fig. 1B with a range between 40 and 90 dB. The study was performed on a guinea pig.

In our first studies we did not assess the phase displacement of CM according to the intensity of the stimulus. We only pursued the possibility of determining an audiometric profile objectively. Currently, objective cochlear microphonic audiometry having been achieved, we have approached other subjects, such as the study already undertaken of objective recruitment,6 determining the masking effect and auditory fatigue, which are pending publication, plus the latency of sensory receptors which we are dealing with currently. The few references for the behaviour of the outer auditory hair cells is probably due to the fact that the procedure we use is not very widespread and to the lack of interest in studying CM, which became established when the difficulties obtaining them and subsequently using them in the clinic were determined.

We term this latency, which is extremely short, measured in microseconds, microlatency. The aim of our contribution is to demonstrate that hearing sensory receptors are bioelectrically active. Nowadays their functional activity is acknowledged, as an essential factor towards understanding the extraordinary frequency selectivity of hearing, compared to the simple analysis of the passive filter according to the theories of von Bekesy.7-10 The active participation criterion of other structures in selective acuity is currently being followed by numerous researchers.11,12

Materials and Method

Material

We used equipment specially designed for the study of CM already widely described in other articles.11-17 The equipment is framed in white in Fig. 2.

We have a stimulator (Fig. 2-1) which generates audiometric tones: 250, 500, 1000, 2000 and 4000 Hz and stimulus for the averaging process, with a pulse every wave cycle of the stimulus. In another unit (Fig. 2-2) we have the amplifier system which is tuned very finely with the frequencies used. The computer (Fig. 2-3) has a suitable programme to average the signal and present the results.

The stimulus generated has to be a pure tone for the CM response to be a faithful copy of the sine wave on which the phase can be studied. This form of stimulus enables the use of an amplifier which is finely tuned to each frequency and thus reject lateral perturbations.

We used the experimental animals that we use currently: rats, guinea pigs and chickens. Here we focussed principally

Figure 1  (A) First CM trace in humans. (B) Trace in guinea pig. Phase displacement is shown in both.
on Wistar rats and only controls were performed on guinea pigs and chickens.

Method

We worked in a closed acoustic field, the only way to stimulate both ears separately. In a free field the slightest alteration of distance or angle invalidates the measurement of the phase displacement.

We sent the sound pressure through a tube which we coupled to the auditory chamber. Each species requires appropriate adaptation. In rats and guinea pigs, which have an auditory canal, the tube is coupled using a specifically sized and shaped part which is placed into the external auditory canal. In birds, which do not have a chamber or canal, we use a flat-based tube for coupling which adheres to its outer surrounds. To do this we used a minimum amount of adhesive on the flat rim which sits on the skin around the orifice and ends in the tympanum.

Before performing the study we checked that the instrumentation as a whole at no time introduced phase alterations. We checked its behaviour using a CM stimulator comprising a dynamic microphone and a resistance network which situate their response at the level of the CM (Fig. 3).

In the traces from the stimulator, at different sound intensities, not the slightest phase displacement was recorded. Consequently, the phase variation observed in the animals is biological in nature, not electronic or mechanical.

The one metre acoustic tube can introduce delay or advancement changes in the stimulus phase if its length is changed. This critical circumstance must be taken into consideration during the exploration. In order to check that the distance between the transducer and the tympanum has not changed, the first determination needs to be repeated and must give an identical result.

Execution:

Anaesthesia:

- Rats: 8% chloral hydrate intraperitoneally.
- Birds: ketamine 30 mg/kg in weight and xylacine 1.35 mg/kg intramuscularly.

Placing of electrodes:

Three electrodes were placed, one on the vertex and the other 2 opposite both mastoids. We used needles with a penetration limit of 3 mm. The test can be performed with contact electrodes, but that would make the process longer as the contact area would have to be shaved. If this technique is transferred to humans it can be done with contact electrodes alone.

When the animal was asleep, the coupling parts to the acoustic tube connected and the electrodes placed, we proceeded to perform the study.

1. We chose 4000 Hz as the maximum working frequency on our current equipment to obtain the greatest precision in measuring the time of latency. Bear in mind that we were averaging each stimulus wave. Therefore we were carrying out a study on 4000 complete waves per second.
2. We gave the lowest sufficient stimulus, generally 70 dB, to obtain an obvious microphonic response.
3. We stored the response.
4. We gave new stimuli at higher levels, 80, 90, 100 and 110 dB.
5. We measured the phase displacements on each increase and calculated the total advancement time of the CM phase. The measurement was taken from the crossing on the graph through line “0” corresponding trace at 110 dB. At 4000 Hz the time we estimated for a complete wave was 250 μs.

We made the calculation on a half wave, which corresponds to a time of 125 μs. The microphonic responses at 70, 80, 90, 100 and 110 dB shifted their position.

Displacements in time due to phase advancement were measured on the corresponding graphs.
In Fig. 4 we present the results obtained in one of the rats used. The intervals marked “a” correspond to the total phase displacement, which can be measured at the start or exit of the half wave or at its point of maximum elongation.

The speed of sound varies in different media. In air it was 340 m/s and in water 1500 m/s. It also varies with temperature and in water with the concentration of solutes. Consequently determining the real time of microlatency depends on the speed of sound propagation inside the cochlea. The values obtained for any medium can be mathematically extrapolated to other propagation speeds without the recording of the latency of the MCs losing validity.

We only considered phase displacement and not amplitude increases of the CM. A dynamic amplitude compression technique is used which does not affect the response phase. The variation of the phase in changes of sound pressure can be clearly observed in the traces.

**Results**

Descriptive statistics:

Table 1 represents the different descriptive statistics of the various sound intensities between 70 and 100 dB versus time (microseconds).

The intensity of 110 dB was taken as a reference (point 0 or of origin) of minimum latency studied and the minor differences in time from the remaining stimuli were taken from this.

Figs. 5 and 6 show the different intensities (in dB) versus time (in microseconds) for each of the ears corresponding to a mean of 10 rats. We can see how on the “right ear” graph the standard deviations are lower than on the “left ear graph”. This might be because:

1. The initial study of the microphonics of the right ear influenced the subsequent study of the left ear. This question was disregarded in our work on auditory fatigue.
2. The couplers to the external auditory canal were arranged differently in both ears and affected how the data were obtained.
Furthermore, on applying the parametric test statistics to check whether the right ear and the left ear were independent we find that:

1. It is implied from Levene’s test that the variances are equal.
2. It is implied from the student’s t-test that the means are equal.

Assuming the above premises and using one way ANOVA we can take both ears as equal and not independent.

**Fig. 7** “both ears” represents intensity (decibels) versus time (microseconds).

We can observe visually how both ears can be taken as a single sample for statistical purposes.

On this basis, we shall use a single sample (RE+LE) of No. = 20. For descriptive statistics we shall use the data from the ear column (both) which is shown in **Table 2**.

Graph 4 represents the different intensities (decibels) versus time (microseconds) for both ears.

It can be observed how at lower intensities the response time is greater, following a proportionality as we reduce this intensity (100–80 dB), and the standard deviations (in microseconds) are greater as the sound intensity decreases (in decibels). Applying specific parametric tests, one-way ANOVA* complemented with Bonferroni posthoc test, the comparisons between the different means are significant ($P^{<.01}$) for 100–90 dB, 90–80 dB, but not for 80–70 dB. At low intensities the noise of the apparatus (background noise) could influence the measurements and therefore the difference between 80 and 70 dB is not significant. The phenomenon has been recorded with other lower frequencies. We show only the results in rats, although microlatency can also be seen in chicken and guinea pigs but of a different magnitude.

**Discussion**

We have found no references with regard to CM latency, only Davis’ assertion of non-existence in the sensory receptors. This assertion, which is currently accepted, is an error which affects the basic concepts of hearing physiology.

Davis establishes his conclusion with different methodology, corresponding to 1976 and therefore it is not appropriate to make a comparative review.

In the works reviewed on auditory latency the hearing potentials obtained in electro-cochleography are considered to be of shortest latency, between 1 and 10 ms, whereas the latency we found for hair cells is measured in microseconds. Very short opening and closing times were also found in studies on ion channel closure in animal experiments using micropipettes, around 40 μs, which vary with temperature, not with the intensity of the stimulus. Nonetheless, work in this regard may bear a relation with CM microlatency.

**Table 1** Descriptive Statistics for Both Ears at Different Sound Intensities (70–100 dB) Versus Time (Microseconds).

<table>
<thead>
<tr>
<th>Intensity* (dB)</th>
<th>Ear</th>
<th>No.</th>
<th>Mean</th>
<th>SD</th>
<th>Standard Error</th>
<th>95% Confidence Interval</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Limit</td>
<td>0.90</td>
<td>15.66</td>
</tr>
<tr>
<td>100</td>
<td>Right</td>
<td>10</td>
<td>6.81</td>
<td>5.06</td>
<td>1.60</td>
<td>3.19</td>
<td>10.42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>10</td>
<td>7.14</td>
<td>7.78</td>
<td>2.46</td>
<td>1.57</td>
<td>12.71</td>
<td>-0.90</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>20</td>
<td>6.97</td>
<td>6.39</td>
<td>1.43</td>
<td>3.98</td>
<td>9.96</td>
<td>-0.90</td>
</tr>
<tr>
<td>90</td>
<td>Right</td>
<td>10</td>
<td>15.36</td>
<td>6.26</td>
<td>1.98</td>
<td>10.88</td>
<td>19.84</td>
<td>9.64</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>10</td>
<td>16.33</td>
<td>9.39</td>
<td>2.97</td>
<td>9.61</td>
<td>23.04</td>
<td>5.42</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>20</td>
<td>15.84</td>
<td>7.78</td>
<td>1.74</td>
<td>12.20</td>
<td>19.48</td>
<td>5.42</td>
</tr>
<tr>
<td>80</td>
<td>Right</td>
<td>10</td>
<td>25.02</td>
<td>7.45</td>
<td>2.36</td>
<td>19.69</td>
<td>30.35</td>
<td>13.55</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>10</td>
<td>26.57</td>
<td>9.09</td>
<td>2.88</td>
<td>20.06</td>
<td>33.07</td>
<td>15.96</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>20</td>
<td>25.79</td>
<td>8.13</td>
<td>1.82</td>
<td>21.99</td>
<td>29.60</td>
<td>13.55</td>
</tr>
<tr>
<td>70</td>
<td>Right</td>
<td>10</td>
<td>30.18</td>
<td>8.04</td>
<td>2.54</td>
<td>24.43</td>
<td>35.93</td>
<td>17.47</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>10</td>
<td>30.02</td>
<td>9.54</td>
<td>3.02</td>
<td>23.19</td>
<td>36.85</td>
<td>19.88</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>20</td>
<td>30.10</td>
<td>8.59</td>
<td>1.92</td>
<td>26.08</td>
<td>34.12</td>
<td>17.47</td>
</tr>
</tbody>
</table>

**Table 2** Sample (RE+LE) of No. = 20. For Descriptive Statistics.

<table>
<thead>
<tr>
<th>Intensity (dB)</th>
<th>No.</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>20</td>
<td>6.97</td>
<td>6.39</td>
</tr>
<tr>
<td>90</td>
<td>20</td>
<td>15.84</td>
<td>7.78</td>
</tr>
<tr>
<td>80</td>
<td>20</td>
<td>25.79</td>
<td>8.13</td>
</tr>
<tr>
<td>70</td>
<td>20</td>
<td>30.10</td>
<td>8.59</td>
</tr>
</tbody>
</table>
and remain consistent with that which is required for the
perception of the highest frequencies.

The procedure used for the measurement of time by
phase displacement is only useful in physical applications. It is
understandable that it is not used in biology, and the
measurements must be made on sine wave signals of longi-
tudinal propagation, on which the frequency and speed of
displacement can be measured depending on the medium.
These conditions only arise in the mechanoelectrical trans-
duction response of the outer auditory hair cells.

This exceptional circumstance is engendering expecta-
tions on our part that instruments and new lines of research
will be developed.

The majority of studies reviewed always use click and
broadband amplifiers, appropriate for the responses sought,
but inadequate for the microphonic response of outer audi-
tory hair cells.

Conclusions

CM are not the product of a simple electromechan-
transduction process. Other biological factors intervene which
regulate their presentation. The time difference in the
appearance of microphonic potentials is obvious, depending
on the intensity of the stimulus, a condition which is
common to all biological latency processes. At present we
should not risk any functional aspect to the microlatency
of hearing receptors, although it appears that theories on
the active function contribution of frequency selectivity are
reinforced. The study of response latency at supracochlear
levels is important for audiological diagnosis. It could be that
one day the latency of the auditory sensory receptors may
provide important information on the delicate and complex
cochlear function.

Conflict of Interests

The authors have no conflict of interests to declare.

References

4. Lempert J, Meltzer PE, Wever EG, Lawrence M. Thecochlo-
6. Sanjuán J. Recruitment y microfónicos cocleares. Acta Otorrin-
11. Bray CW. The nature of acoustic response: the relation between
sound frequency and frequency of impulses in the auditory
12. Bray CW. Action currents in the auditory nerve in response to
15. De los Santos G, Sanjuán J, Gavilán J. Potenciales microfó-
nicos cocleares con electrodos de superficie en el diagnóstico
17. Sanjuán J. Estudio de la audición en prematuros. Microfó-
18. Gueuning F, Varlan M, Eugene C, Dupuis P. Accurate distance
measurement by an autonomous ultrasonic system combining
time-of-flight and phase-shift methods. In: Instrumentation and
Measurement Technology Conference, 1996. IMTC-96. Confer-
ce Proceedings. ‘Quality Measurements: The Indispensable