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Impact of auditory stimulation at a frequency of 5 Hz in verbal memory

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Introduction. The objective of this study was to establish whether stimulation at 5 Hz enables immediate words recall.

Method. A total of 20 participants received auditory stimulation at 5 Hz-theta, beta-13 Hz frequencies, white noise (WN) and words.

Results. The results indicate significant differences in the number of recalled words per day depending on the stimulation frequencies. From the third to the fifth day a significant association was shown between increasing numbers of recalled words at 5 Hz compared with the rest of the frequencies. If we take the number of words recorded during the recording of the magnetoencephalography into account, significant differences with greater numbers of words between 5 Hz and between 13 Hz and 5 Hz and WN were found in the second measure. The median frequency only showed significant differences in stimulation at a frequency of 5 Hz.

Conclusion. Auditory stimulation over a long time at a frequency of 5 Hz generates a coupling of brain activity that increases the capacity of immediate verbal memory.

Key words:
Verbal memory. Theta rhythms. Auditory stimulation.

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Efecto de la estimulación auditiva a una frecuencia de 5 Hz en la memoria verbal

Introducción. El objetivo de este estudio es establecer si la estimulación a 5 Hz favorece el recuerdo inmediato de palabras.

Método. Veinte participantes recibieron estimulación auditiva a frecuencias de 5 Hz-theta, 13 Hz-beta, ruido blanco (RB) y palabras.

Resultados. Los resultados indican diferencias significativas en número de palabras recordadas por día entre las frecuencias. A partir del día 3 hasta el día 5 se observó una asociación significativa entre un mayor número de palabras recordadas con 5 Hz comparadas con las demás frecuencias. Si tenemos en cuenta el número de palabras recordadas durante el registro en la magnetoencefalografia, en la segunda medida se encontró diferencias significativas con mayor número de palabras entre 5 Hz y 13 Hz y entre 5 Hz y RB. En la frecuencia mediana sólo se presentaron diferencias significativas en estimulación a una frecuencia de 5 Hz.

Conclusión. La estimulación auditiva durante largo tiempo a una frecuencia de 5 Hz genera un acoplamiento de la actividad cerebral a dicho ritmo que aumenta la capacidad de memoria verbal inmediata.

Palabras clave:
Memoria verbal. Ritmos theta. Estimulación auditiva.

INTRODUCTION

The external auditory stimulation has been considered as a way of cortical rhythm induction from the 1930's until the present date[1-6]. On the other hand, verifying how certain cognitive functions modify the brain rhythms has been a classical subject of study within psychophysiology[7]. Within these rhythms, it seems that the theta band is that which has been most associated with the basic processes associated with memory. Some investigators have found a considerable increase in the theta band power in selective attention processes[8], during bimodal sensory stimulation on the frontal level[9], during the coding processes and memory recall[10-12], during meditation stages in which there is a positive emotional state and profound attention[13,14]; and in the cognitive integration processes, association of functions and response control[3].

It also appears that the theta band has extensive activity in the brain[11,15], mainly in the hippocampal system[16], and is considered basic for memory. However, the most convincing evidence on the relationship between theta activity and

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memory comes from animal research, specifically on the relationship between theta activity and coding of new information in the memory.

Different studies have demonstrated a close relationship between synchronic activation in theta band and increase of long-term potentiation (LTP)[17] that would occur in several cortical regions, but especially in the hippocampus. The intensity of the LTP increases linearly with increase of theta power, which has direct consequences on learning.

In spite of the above, the possibility of maintaining a specific cerebral rhythm in the brain by external auditory stimulation is very limited in time, the findings still being inconsistent[2-4,18] although others[3] have be able to increase the power of the brain rhythms thanks to audio-visual stimulation. Timmermann et al.[4] demonstrated that repeated stimulation during several 20 min sessions would produce changes in the distribution of brain frequencies that could last up to 30 min.

In view of these data, on the one hand we propose inducing the brain to a state of stable theta activity and on the other, to verify if the efficacy of the memory process increases during this period of predominant theta activity, compared with a spontaneous brain activity state.

METHOD

Subjects

A total of 18 subjects (4 men and 14 women), students and administrative workers, with a mean age of 29.83 years and standard deviation of 11,807, and minimum and maximum of 20-56 years, participated voluntarily in the study. The general cognitive state was evaluated with the Mini-Mental State Examination[19] with an X: 29.83, and SD: 0.383. Immediate verbal memory was measured with the subtests of Wechsler III Memory Scale[20] of the word list I and II: list A attempt I (X: 6.781; SD: 0.478), total recall score (X: 38.11; SD: 4.028), list B (X: 6.39; SD: 2.110), short-term response (X: 10.50; SD: 1.339), pending learning (X: 4.39 (0.1378), recall of list A, 25 min (X: 10.22; SD: 1.478), total recognition score (X: 23.72; SD: 0.461) and digits (X: 17.94; SD: 3.670). All the participants were within normal limits and had given their written informed consent before being enrolled in the study.

Auditory stimulation

Auditory stimulation consisted in the presentation of a 130 Hz tone with 5 Hz stimulation frequency, another one of 13 Hz and as white noise (WN) control for 15 min, and in the final 2 min, 20 overlapping words had to be memorized.

The words were selected from among a group of 1,917 words of frequent use in the Spanish language[21]. A total of 660 words were taken from this distribution, considering the extension of the word (5, 6 and 7 letters) and the number of words in each group. Randomly, 33 groups of 20 words with intervals of 2, 3, and 4 seconds were formed.

The frequency tones of 5 Hz, 13 Hz, WN were generated with the Adobe Audition software. The recordings were made in stereo at 22.050 Hz and 16 bits. The words were recorded with 20 dbs and 15 dbs dynamics, the with a duration of each word being from 500 and 1,700 ms. Volume of the recordings were normalized at 12 dbs for the words and at 18 dbs for the frequencies.

Auditory stimulation was made under two situations, a so-called conventional one and the other in recording of cerebral activity with magnetoencephalography (MEG). The stimuli were presented with the Windows Media Placer program for the conventional stimulation and a KME® SPA240S amplifier was used to record the brain activity in MEG. The stimuli were presented at an intensity of 80 dbs.

Procedure

The participants were subjected to three types of auditory stimulation (5 Hz, 13 Hz, WN) under the two conditions: conventional and MEG recording. First, the MEG was recorded in session 1, then they received 9 sessions under conventional condition and finally another MEG recording was performed in session 11 for each type of stimulation. At the end of each session, the words recalled were recorded to evaluate immediate memory. To do so, each participant had to verbally repeat the words they recalled. Each participant attended two sessions per workday, for 15 days, from Monday to Friday. The sessions were changed from one type of stimulation to the other after a separation of one weekend.

The conventional stimulation was made in a soundproof room with faint light, using a device consisting in stereophonic headphones connected to a computer for the presentation of each session. The participants were instructed to remain comfortably seated in a chair and were informed that they were going to receive a stimulation for 15 min where they would continuously listen to a noise and finally some words that they should memorize to be recalled immediately.

The MEG recordings were made with the participants in a room that had been isolated for the environmental magnetic fields, using a «whole-head» system of 148 Magnes 2500 WH® channels (4D Neuroimaging, San Diego, CA). During the recording, the participants remained in a supine lying position, with their head inside a helmet-shaped sensor. The participants were instructed to remain aware and immobile while the recording was being performed and they were informed that there were going to be controlled with a video system. The electroencephalogram, electrocardiogram and electrooculogram were recorded simultaneously. All the da-

ta were obtained using a sampling rate of 254.31 Hz and a band-pass filter of 0.1-100 Hz.

The content of each one of the sessions was the following: a first recording of 5 min long spontaneous MEG activity in which the subjects were completely at rest and did not receive any type of stimulation. Once the spontaneous recording was made, the participants underwent a second 15 min long recording of the MEG activity with the session.

In the MEG session, all the stimuli (frequency and words) were presented binaurally through two 5-meter long plastic tubes that ended in disposable adapters that were inserted into the subject's ear.

Analysis of the MEG signal

The magnetic brain activity of the subjects under study was analyzed with the median frequency of spectrum parameter. This measurement made it possible to characterize the MEG frequencies spectrum in an easy way, since it is defined as the frequency under which 50 % of the total energy of the signal is found. Consequently, this variable summarizes the global shift of the spectrum towards high or low frequencies[22]. In this study, the signal spectrum was calculated from the Fourier Transform of the self-correlation function of the MEG registries. Previously, the recordings were visually reviewed by an expert and the recordings corresponding to 11 of the 18 subjects ruled out as they presented great contamination due to artifacts. Thus, the median frequencies were only calculated in the MEG recordings of 7 subjects. For each one of these subjects, the spectrum of the signal was estimated in each one of the 148 channels and then these were characterized by the median frequency, obtaining 148 values per subject. To simplify the analyses, the average of the median frequency values of these 148 channels was calculated to obtain a mean value per subject, stimulation frequency and session.

RESULTS

The fundamental purpose of this study is to verify the effect that auditory stimulation on different frequencies (5, 13 Hz and WN) has on immediate free word recall. As has already been described, the presentation of the sessions was made under different experimental conditions in the MEG recording and under conventional condition and the analyses of the data were made independently.

The memory data obtained during the MEG recordings were analyzed with the ANOVA 3 × 2 (frequency × session) for repeated measures. The independent variable of frequency has three levels, represented by the three stimulation frequencies that the subjects receive: 5, 13 Hz and WN. The variable of independent session is made up of two levels that correspond with the two times in which the recordings are done: the first session of recordings in MEG called «baseline» and the second one of recordings after the last recording.

The data obtained under the conventional situation were analyzed with an ANOVA 3 × 5 (frequency and day of estimation). The variable of frequency is identical to that described previously. The variable of day of stimulation is made up of 5 levels that were obtained by averaging the number of words recalled in the two daily sessions (days 1, 2, 3 and 4), the average of day 1 was called baseline and session 10 the last session (day 5). The significance level for the principal contrasts was $p < 0.05$. However, this significance level was corrected following Bonferroni's criterion for all the tests that involved multiple comparisons. Thus, the significance level of the ANOVA was evaluated with the Huynh-Feldt correction whenever it was appropriate as a precaution against the inhomogeneities of the variances.

The MEG values of median frequency corresponding to each stimulation frequency were analyzed separately using the Student's *t* test. All the statistical analyses were done with the SPSS for Windows version 10.0.

Memory under conventional situation

The number of words recalled during the «conventional» situation is influenced by the principal effect of the frequency factor ($F2.34: 61.981$; $p<0.0001$) and by the interaction of frequency × day of stimulation ($F8.136: 2.909$; $p<0.01$). In this case, the frequency variable shows a clear significant effect, so that the 5 Hz frequency is associated with a greater number of words recorded independently of the stimulation day, both when compared with 13 Hz ($p< 0.0001$) and with WN ($p<0,0001$). If we analyze this effect more precisely through the interaction, we verify two tendencies. In the first place, if we establish the frequency variable, we observe that there is no significant variation, of either an increase or decrease, for any of the stimulation frequencies in regards to the number of words recalled during the days (table 1).

Table 1 shows how the number of words becomes modified day by day in each frequency, but these modifications are not significant. However, if we establish the variable of day of stimulation, the panorama is quite different. There are no significant differences on day 1. On day 2, there are significant differences between 5 Hz and 13 Hz ($p<0.01$) and between WN and 13 Hz ($p<0.01$). In both cases, the frequency of 13 Hz is associated with a lower number of recalled words. After day 3, there is a clear tendency, by which 5 Hz is associated with a greater number of recalled words, in comparison to both 13 Hz ($p<0.05$) and WN ($p<0.001$). The same occurs on day 4, at 5 Hz-13 Hz ($p<0.0001$), 5 Hz-WN ($p<0.0001$). Again we find 13 Hz associated with a significantly lower number of recalled words than that of WN ($p< 0.05$). Finally, on day 5, the tendency, with significant differ-

Table 1 Word recall by frequency and day in conventional situation

Frequencies	Day	Mean	SD
5 Hz	1	12.05	3.74
	2	11.55	2.41
	3	12.30	2.45
	4	13.08	2.62
	5	12.27	3.15
13 Hz	1	10.86	2.75
	2	9.94	1.93
	3	10.69	2.60
	4	9.69	3.12
	5	10.50	2.79
White noise	1	10.05	2.33
	2	11.69	2.98
	3	9.88	2.11
	4	10.75	2.48
	5	10.83	3.29

ences between 5 Hz-13 Hz ($p<0.05$) and 5 Hz-WN ($p<0.05$), is verified.

In summary, the results indicate an influence of the stimulation frequency in the recognition of words that clearly benefits 5 Hz. The intra-frequency data indicate that 5 Hz is always associated with a greater number of recalled words, but the daily variations are not significant. However, the appearance of inter-frequency differences is gradual and arises clearly after the third day of stimulation.

Memory during MEG recording

As we mentioned in the procedure of the statistical analyses, the memory tasks performed in conventional situation and in the MEG are not comparable due to their different experimental condition, so that they have been analyzed independently. However, if we observe the scores obtained during the first MEG recording, when the subjects receive the first stimulation for each frequency, and we compare them with the data obtained after day 1 of conventional stimulation, we find an increase in the words recalled of 19.18% at 5 Hz versus 7.4% at 13 Hz and 9% for the WN.

The number of words recalled during the MEG recordings are influenced by the principal effects of the frequency factors ($F2,34$: 5.07; $p<0.01$) and session ($F1,17$: 37.00; $p<0.0001$), and by the interaction between both variables ($F2,34$: 7.94; $p<0.01$). The principal effects indicate the tendency towards a greater number of words recalled with 5 Hz, and a global increase of the number of words, independently of frequency, in the second measure obtained during the final MEG recording. However, these effects may be analyzed in much greater detail through the interaction between the factors. In this sense, if we establish the variable session first, we find that there are no significant differences between frequencies in the baseline.

However, in the second measurement, we observed significant differences between 5 Hz and 13 Hz ($p<0.01$), and between 5 Hz and WN ($p<0.01$). In both cases, there was a significantly greater number of recalled words for the 5 Hz frequency. When we established the stimulation frequency, we found that the number of words recalled in the last session was only significantly greater to those recalled in baseline ($p<0.0001$) only at the 5 Hz frequency while there were no significant differences at the other frequencies (table 2).

Regarding percentages, the increase of the words recalled in the last sessions compared to baseline was 33.5% at 5 Hz, 4.89% at 13 Hz and 11.89% with WN.

Median frequency

In the case of the stimulations at 13 Hz frequency and with WN, the results do not show any significant differences between the median baseline frequencies and those corresponding to the last session ($p=0.470$ and $p=0.640$, respectively). On the contrary, regarding the stimulation on the theta band with a 5 Hz frequency, there are significant differences between the median frequency of both sessions ($p<0.05$). This shift of the spectrum towards lower frequencies produced by the stimulation at 5 Hz can be seen in Table 3. This table shows the values of the median frequency associated to each type of stimulation and session.

Furthermore, the shift caused by stimulation at 4 Hz is shown in figure 1. This figure shows the distribution of the median frequency values for a subject at the onset and end of the theta band stimulation. It can be observed that this shift of the spectrum towards lower frequencies results in lower values of median frequency corresponding to the final session of this stimulation.

Table 2 Word recall by frequency and MEG recording session

Frequencies	Session	Mean	SD
5 Hz	Baseline	10.11	3.08
	Last session	13.00	3.39
13 Hz	Baseline	10.12	2.78
	Last session	10.38	2.78
White sound	Baseline	9.22	2.31
	Last session	10.16	2.87

Table 3 Median frequencies by frequency of stimulation and session

Frequency	Session	Mean	SD
5 Hz	Baseline	8.31	1.66
	Last session	5.82	2.23
13 Hz	Baseline	7.89	1.26
	Last session	7.26	1.85
White sound	Baseline	7.02	2.29
	Last session	6.49	1.86

DISCUSSION

The results found indicate that auditory stimulation at 5 Hz frequency is associated with a greater number of recalled words and with a decrease of the MEG frequency close to the 5 Hz. There is also a progression during the auditory stimulation at 5 Hz in the improvement of word recall after the third session while the stimulation at 13 Hz worsens the capacity to recall words and the WN maintains the number of words recalled stable.

The fact that verbal recall improves with stimulation at 5 Hz could be related to the data obtained by other investigators who justify the increase of the theta band power during the coding and recall processes[10-12]. This relationship between 5 Hz and memory could be associated to short-term storage work processes, manipulation and use of mental representations[3], since the subject was asked to recall the words immediately after hearing them. Krause et al.[24] reached observed results as they found an increase in the theta band in the interval in which the processes related with working memory are active. The results could also be justified from the pathology of the memory given that studies with patients having mild cognitive deterioration show a decrease in the theta band both during baseline stages and during memory tests. This is probably a consequence of the neuronal loss in structures that are very important for memory and in the hippocampus[25,26].

A surprising result is the fact that the auditory stimulation at 13 Hz makes recall worse. This outcome could be interpreted in different ways. On the one hand, this frequency could be associated with another type of cognitive functions, such as early attention, task complexity, memorization of multiple stimuli and new stimuli, among others[27-31], or, it could be due to blockage of the theta effect on learning[11]. We could also consider this frequency as an interference in the coding process and recall in favor of the 5 Hz, where data are found that justify an increase of the theta band power during coding and recall[10-12].

The results verify the effect of the auditory stimulation at 5 Hz over a long period, shown by a significant decrease of the median frequency of the baseline brain rhythms. This decrease tends to move the median frequency closer to values close to the 5 Hz, which does not occur with the stimulation at 13 Hz. Some investigators have been able to increase brain rhythms close to those of the stimulation frequency with audiovisual stimulation[3,4] while others have not been able to find significant differences between the at rest states and external sensorial stimulation in another type of frequencies related with the relaxation states[18]. In fact, Rosenfeld et al.[32] found effects during audiovisual stimulation at a frequency of 10 Hz with an increase of the alpha rhythm frequency. That study also did not find any significant effect with the auditory stimulation at 13 Hz nor did Teplan et al.[3] with audiovisual stimulation, and they only found an increase in power in the theta and alpha bands. These results could be due to the fact that the

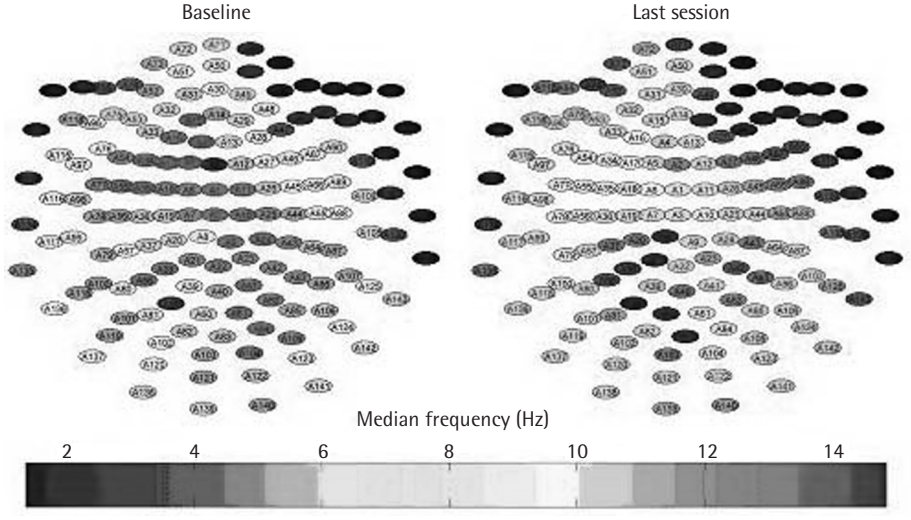

Figure 1 *Distribution of values of median frequency in a subject in the first and last session with stimulation at 5 Hz.*

beta rhythm could be associated with another type of cognitive activities other than memory and that are more complex with greater capacity and integration of many more brain areas[27-29].

The association between brain rhythm at 5 Hz and the increase of the number of words during the MEG recording when we compare the first and last sessions makes it possible to understand that there is a direct relationship between the brain rhythm of 5 Hz and better immediate verbal recall capacity. It can be observed that this function is significantly altered in patients with mild cognitive deterioration. In this sense, different investigations have found a decrease of the theta rhythm during a working memory task[33], an association between hippocampal atrophy and the decrease of the theta band power during the cognitive processes[25,26] and other consider that it could predict a decline of cognitive functions in patients with mild cognitive deterioration[34].

It can be deduced from our results that regular training with auditory stimulation of tonal frequencies could generate significant changes in the brain functioning associated with improvement of cognitive functions[3,4,35,36].

In conclusion, we consider that long-term auditory stimulation at a frequency of 5 Hz generates a coupling of brain activity to said rhythm that increases immediate verbal memory capacity.

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