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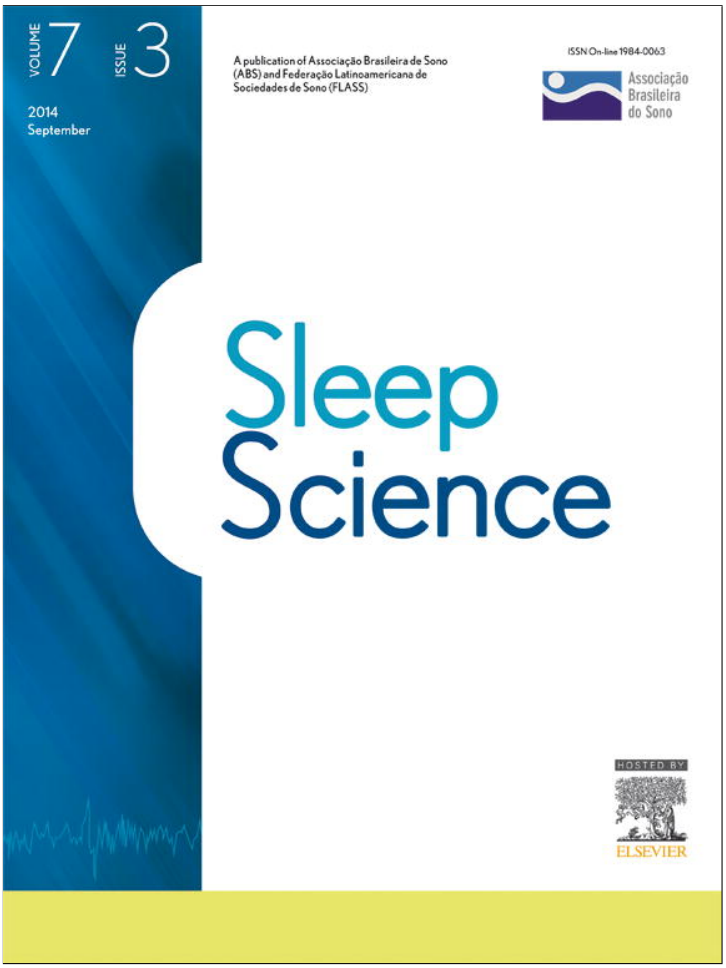

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Sleep Science 7 (2014) 143–151

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The Impact of Sound on Electroencephalographic Waves during Sleep in Patients Suffering from Tinnitus

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ARTICLE INFO

Article history:
Received 16 April 2014
Received in revised form
6 August 2014
Accepted 11 August 2014
Available online 5 October 2014

Keywords:
Tinnitus
Electroencephalography
Sound stimulation
Sleep
Coherence
Power spectra

ABSTRACT

Based on the knowledge that sensory processing continues during sleep and that a relationship exists between sleep and learning, a new strategy for treatment of idiopathic subjective tinnitus, consisted of customized sound stimulation presented during sleep, was tested. It has been previously shown that this treatment induces a sustained decrease in tinnitus intensity; however, its effect on brain activity has not yet been studied. In this work, we compared the impact of sound stimulation in tinnitus patients in the different sleep stages.

Ten patients with idiopathic tinnitus were treated with sound stimulation mimicking tinnitus during sleep. Power spectra and intra- and inter-hemispheric coherence of electroencephalographic waves from frontal and temporal electrodes were measured with and without sound stimulation for each sleep stage (stages N2 with sleep spindles; N3 with slow wave sleep and REM sleep with Rapid Eye Movements).

The main results found were that the largest number of changes, considering both the power spectrum and wave's coherence, occurred in stages N2 and N3. The delta and theta bands were the most changed, with important changes also in coherence of spindles during N2. All changes were more frequent in temporal areas. The differences between the two hemispheres do not depend, at least exclusively, on the side where the tinnitus is perceived and, hence, of the stimulated side. These results demonstrate that sound stimulation during sleep in tinnitus patients' influences brain activity and open an avenue for investigating the mechanism underlying tinnitus and its treatment.

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1. Introduction

While all sensory processing persists during sleep, the auditory input is particularly relevant for continuously monitoring the environment [1,2].

Several treatment strategies of tinnitus are based on sound stimulation and evidence indicates that they are more effective if sound mimics the tinnitus [3]. All these protocols conduct sound stimulation during the day, while patients are awake. Based on the knowledge that auditory processing continues during sleep [2] and that a relationship between learning and memory and sleep stages has been established, our group has embarked a new strategy for the treatment of idiopathic subjective tinnitus. A protocol of customized

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http://dx.doi.org/10.1016/j.slsci.2014.09.011
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sound stimulation during sleep has resulted in a decrease in the subjective intensity, significantly improving the patients' quality of life [4].

It has been argued that slow wave sleep is important for declarative memory and that working memory is processed mainly during REM (Rapid Eyes Movements) sleep; however, the integrity and interaction of the different sleep stages for learning and memory process have also been suggested [5–8]. Slow EEG oscillations (less than 1 Hz) have been involved in the consolidation of long-term memory [9] and in the homeostatic regulation of synaptic connections [10]. Rhythmic acoustic stimulation induces K-complexes, which are considered a "forerunner" of slow oscillations in slow wave sleep stage [6,11]. Slow oscillation during slow wave sleep promotes consolidation of memory and the post-sleep facilitation of encoding new memories [12]. Slow waves may be modulated by low-frequency auditory stimulation [13]. Studies with functional magnetic resonance showed that auditory cortical activity is maintained during sleep but varies with stimulus significance [14,15].

The goal of this study was to explore the changes on brain activity induced by the sound stimulation during sleep in tinnitus patients.

2. Material and methods

2.1. *Polysomnographic recording*

Ten patients with idiopathic tinnitus, treated with sound stimulation mimicking tinnitus during sleep were studied [4]. The inclusion criteria were (1) adult patients with unilateral or bilateral subjective idiopathic tinnitus, (2) evolution of more than 6 months and (3) Tinnitus Handicap Inventory score above 17. The exclusion criteria included patients that demonstrated (1) objective or subjective secondary tinnitus, (2) hearing loss of 50 dB hearing threshold level (HTL) or worse in more than one frequency of the audiogram, (3) those that had undergone other treatments for tinnitus in the past year, (4) current use of hearing aids, (5) use of psychoactive drugs, (6) depression (Hamilton scale test above 13), and (7) sleep disorders not related to tinnitus (apnea, periodic limb movements, narcolepsy, etc.).

All of them were recorded with a complete Polysomnography throughout the night (patient characteristics are shown in Table 1). Polysomnographic recording were done when patients were habituated to treatment and had the largest decrease in the intensity of tinnitus, in the second or third months of treatment.

The Polysomnography (PSG) was carried out with the usual clinical protocol through computerized Polysomnograph (Bio PC V11/V12, Akonic S.A.), recording 10 electroencephalographic channels (frontals: F3, F4; centrals: C3, C4; parietals: P3, P4; temporal: T3, T4, T5, T6, following the internationally accepted standard denomination), electrocardiogram, electromyogram, eye movements and oxygen saturation. All EEG recordings were monopolar, recorded from scalp electrodes and separate ear electrodes A1 and A2, with electrodes referenced to linked ear lobes. The sampling frequency was set at 256 Hz. The EEG acquisition system is equipped with hardware high-pass filters with cut-off frequency at 0.5 Hz and hardware low-pass filters with cut-off frequency at 100 Hz. In addition, there is a selectable notch filter to suppress 50/60 Hz power line noise. No digital post-processing filters were applied. One researcher accompanied the patient all night, diagnosing the sleep stages online.

After beginning the night with the usual sound stimulation for tinnitus treatment, sounds are stopped after a minimum of one pass through each of the sleep stages: somnolence (stage N1), stage N2 with sleep spindles, stage N3 with slow wave sleep and sleep with Rapid Eye Movements (REM). The rest of the night the patients continue to sleep in silence (Fig. 1). All patients were started with sound stimulation because they are habituated and improve sleep onset with the sound. This enhances the disturbances caused by tinnitus, e.g., anxiety, increased sleep latency, awakenings, shallow sleep the first few hours.

2.2. *Sound stimulation*

Each patient was stimulated with a sound created through special software combining different types of sounds (pure tones, band noises, white noise) designed with the specific aim of being able to match their perception. Each night patients fixed the sound intensity in the same level that feel the tinnitus. These sounds are applied in a continuous way (sound stimulation for each patient is shown in Table 1, third column) [4]. Customized ear buds with flat response in the range of 0.125–16 kHz were created for each of the patients studied. These ear buds provide a reliable output, are comfortable for the patient and ensure a fixed distance between the source of sound and the eardrum. The specific sound was loaded onto the patient's devices (iPod Touch). The whole system output was measured using an artificial ear (Ear Simulator 43AC, GRAS sound and vibration) and a sound level meter (Bruel & Kjaer type 2250) and calibrated with a sound calibrator (Bruel & Kjaer type 4231@1 kHz, 94 dB SPL).

2.3. *Data processing*

Twenty temporal windows (2 sec duration each one) were selected in each sleep stage (N2, N3 and REM); 10 of them during silence and the other 10 during sound stimulation. Always data were compared in the same patient.

We analyzed the power spectra and the coherence in electroencephalographic waves recorded by electrodes F3, F4, T3 and T4. We compared the power spectra during noiseless (as a "Control") versus sound stimulation, exploring different electroencephalographic frequency bands (delta: 0.5–3.5 cps; theta: 4–7.5 cps; alpha: 8–12 cps) in the same sleep stage. A comparison between the left and right hemispheres (T3 vs. T4 and F3 vs. F4) was also carried out.

We studied the wave's coherence percentages, analyzing pair of intra-hemispheric electrodes (F3-T3 and F4-T4) and inter-hemispheric electrodes (F3-F4 and T3-T4). The overall coherence (considering all frequencies, from 0.5 to 12 cps, together) and the coherence of each range of frequency were considered, comparing temporal windows

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Table 1 – Power spectra changes during sound stimulation in each sleep stage.

Patient	dB ↓	Tinnitus intensity and match noise	R–L handed	Stage N2	Stage N3	REM
WF, m 42 y.o.	4	40 dB SPL 4,9 + 9,8 kHz R,L = 43 dB	R	Theta F3↑		Theta F4↑ T3↑
MB, f 64 y.o.	9	60 dB SPL 4,6 + 4,5 + 4,4 + 4,3 kHz L = 60,5 dB	R	Delta F4 ↓ T3↓		Theta T3↓
GB, f 39 y.o.	10	59 dB SPL 7,3 + BN 7,3 kHz R = 54.1; L = 55.1 dB	R	Theta T3↑	Interh delta T Interh theta T	Delta F4↑ T3↑
TL, f 32 y.o.	12	40 dB SPL 3, 6 + 6,6 kHz R,L = 54,1 dB	L	Theta F4↑	Theta F3↑ F4↑ T3↑ Alpha T3↑	Interh alpha T
SO, f 60 y.o.	15	59 dB SPL 11,44+10,44+9,40+9,20 kHz R,L = 69,1 dB	R		Delta T3↓ Theta F4↓	
FC, m 58 y.o.	19	68 dB SPL 8,0 + BN 10,7 kHz R,L = 67,4 dB	R		Theta F3↑	
MA, m 21 y.o.	21 *	59 dB SPL 7,5 + 15,1 kHz L = 46.1 dB	L	Interh theta T		
EC, f 32 y.o.	21	62 dB SPL BN 3,5 kHz R = 44,1 dB	R	Alpha T4↓		Delta F4↑ Theta T4↑ Interh delta F
EP, m 32 y.o.	22 *	53 dB SPL 6,6 + 6,8 kHz R = 59.9; L = 65.9 dB	L		Alpha T4↓	
LG, f 55 y.o.	30	60 dB SPL 6,1 + 6,2 + 6,5 kHz R = 56,9; L = 63 dB	R	Delta T4↑ Theta T4↑ Alpha F3↑ T4↑	Delta T4↑	

Table 1 Shows statistically significant power spectra changes in each sleep stage, comparing delta, theta and alpha bands power spectra during silent and sound stimulation. Different sleep stages were analyzed separately (Stage N2; stage N3 and REM, rapid eye movements). From left to right: patient identification; m, male; f, female, age (y.o.); dB↓, tinnitus intensity decrement at the end of the treatment (★patients in whom the perception of tinnitus disappeared); Tinnitus intensity and "Match noise", tinnitus intensity before treatment (15 days average in dB SPL); "Match noise", characteristics of the created sound for stimulation, "BN", Band Noise; "R" and "L", right and left stimulation side; "F" and "T", frontal and temporal electrodes; "3" and "4", left and right positions, respectively; "Interh" is the comparison between left and right brain hemisphere; ↑ and ↓, power spectra increments and decrements during sound stimulation. Only statistical significant changes are shown. Data were statistically analyzed by one-way ANOVA followed by Tukey post test to compare all pairs of columns.

during silence with temporal windows during sound stimulation.

Signal processing was done using sets of 10 time blocks for each measurement channel. The duration of each time block was 2 sec.

An FFT algorithm was applied to each time block. Sixty complex data (real R(f) and complex I(f) part of the discrete Fourier transform) were obtained for each channel and time block, ranging from 0.5 to 30 Hz with frequency increment of 0.5 Hz.

The interchannel coherence was calculated applying the algorithm:

$$p_i(f) = \sum_{m=1}^{M} \left\{ \left[R(m,\ ch_i, f)\right]^2 + \left[I(m,\ ch_i, f)\right]^2 \right\} \quad (1)$$

$$p_j(f) = \sum_{m=1}^{M} \left\{ \left[R(m,\ ch_j, f)\right]^2 + \left[I(m,\ ch_j, f)\right]^2 \right\} \quad (2)$$

$$Real\ p_{ij}(f) = \sum_{m=1}^{M} \left\{ R(m,\ ch_i, f) \cdot R(m,\ ch_j, f) + I(m,\ ch_i, f) \cdot I(m,\ ch_j, f) \right\} \quad (3)$$

$$Imag\ p_{ij}(f) = \sum_{m=1}^{M} \left\{ -R(m,\ ch_i, f) \cdot I(m,\ ch_j, f) + R(m,\ ch_j, f) \cdot I(m,\ ch_i, f) \right\} \quad (4)$$

where $p_i(f)$ is signal power for channel i, $R(m, ch_i, f)$ and $I(m, ch_i, f)$ are the real and imaginary parts, respectively, of the FFT for channel i, time block m and frequency f. Analogous notation is used for channel j. M is the number of time blocks used. A value of M = 10 was used during data acquisition.

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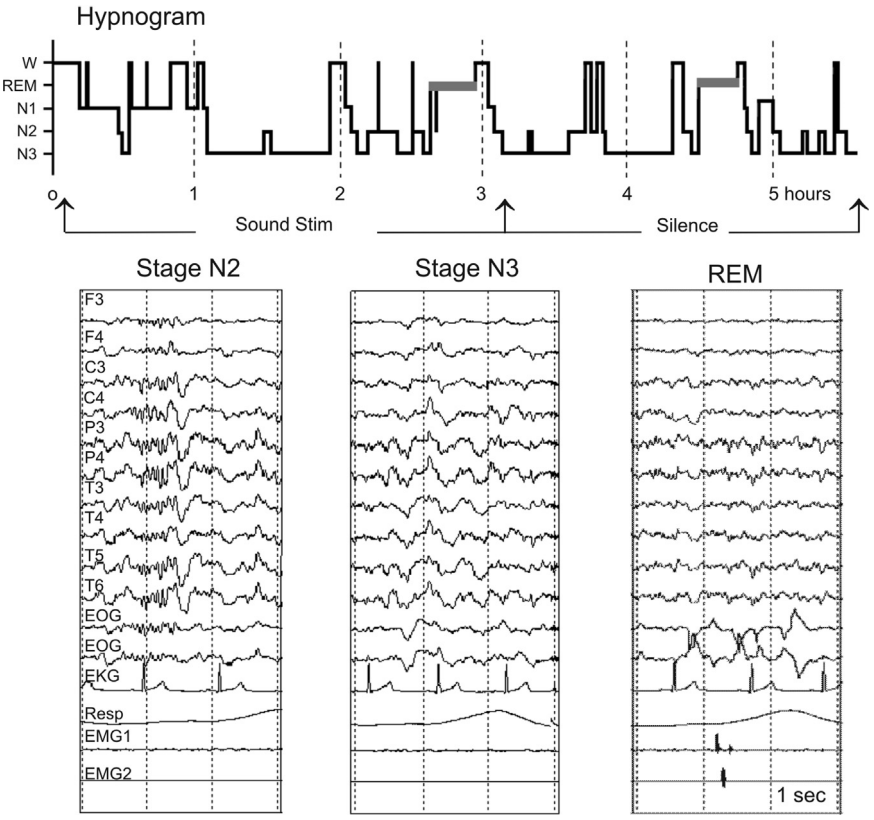

Fig. 1 – Polisomnographic recording of patient L.G. She was recorded nocturnal physiological sleep for almost six hours. From top to bottom, hypnogram showing sequences of the different sleep stage through the night: Wakefulness (W), Rapid Eyes Movement Sleep (REM), stages N1, N2, N3. Sound stimulation was applied in the first half of the night (arrows).The 3 boxes below show 3 sec of different sleep stages: 10 electroencephalographic recordings, 2 electro-oculograms (EOG), electrocardiogram (EKG), respiratory movements (Resp) and legs movements (EMG1 and EMG2).

$Real\ p_{ij}(f)$ and $Imag\ p_{ij}(f)$ are the real and imaginary parts of the cross-spectrum between channels i and j.

Then the coherence between channels i and j was calculated by the formula:

$$Coherence\ (f) = \sqrt{\frac{\left[Real\ p_{ij}(f)\right]^2 + \left[Imag\ p_{ij}(f)\right]^2}{p_i(f) \cdot p_j(f)}} \quad (5)$$

According to Eq. (5), coherence values range from 0 to 1. By multiplying these values by 100, it is possible to express coherence as percentages.

All the results were statistically validated using analyses of variance (ANOVA). Comparisons for power spectra analysis were performed by means of one-way ANOVA followed by the *post hoc* Tukey test. Comparisons involving coherence analysis were done with a Kruskal–Wallis ANOVA followed by the Dunn's multiple comparisons test. P-values below 0.05 were considered statistically significant.

All participants signed their consent after being fully informed of the goal and characteristics of the study. This research was approved by the Medical School Ethical Committee for Human Research of the CLAEH Faculty of Medicine, in accordance with international guidelines for human research.

3. Results

The hypnograms of all patients showed a normal proportion of sleep stages, normal latency to sleep, with physiological ultradian rhythm present, confirming previous results that sound stimulation does not affect sleep cycle. Furthermore, in the clinical interview all patients recognized that with the sound stimulation they were less anxious and better predisposed to sleep [4] (Fig. 1).

3.1. *Power spectrum analysis*

Sound stimulation induced changes in the power spectra of EEG waves during all sleep stages; however, considering each patient only two showed changes in all sleep stages (G.B. and T.L.). Three patients were stimulated monaurally (two of them on the left and one on the right side), in cases where the tinnitus was lateralized. However, changes were not correlated with the stimulation side. Four out of ten patients showed some degree of inter-hemispheric differences, however two of them were binaurally stimulated (Table 1). Regarding sleep stages, 35.5% of statistically significant changes took place during stage N2, 35.5% in stage N3 and the remaining 29% during REM sleep. The 27% of the significant changes were decrements while the 73% showed

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increments in the power spectrum during the sound stimulation. Across all patients and stages, the theta was the band that showed most significant changes (48%), followed by the delta band (36%). The alpha band displayed the smallest change (16%, Table 1). Considering the recording location, temporal electrodes showed larger change than frontal ones (61% vs. 39% respectively, Table 1). Fig. 2 shows an example of the analysis protocol conducted on each dataset. Thirty-six comparisons were performed across 10 temporal windows selected in each sleep stage. The power spectra of delta, theta and alpha bands were analyzed by comparing the periods of silence and sound stimulation at frontal and temporal electrodes (F3, F4, T3 and T4). In this example (Fig. 2) 27 out of 36 power spectra showed increment (75%) being statistically significant for five of them; only one inter-hemispheric comparison was also statistically significant (in REM sleep) in this patient binaurally stimulated.

3.2. *Coherence analysis*

Statistically significant changes were found in both intra-hemispheric and inter-hemispheric coherence in every sleep stage and in all the frequency bands analyzed. Table 2 shows changes statistically significant comparing coherences control (during silence) with periods with sound stimulation. Table 2 also shows the inter- and intra-hemispheric differences that appeared with sound stimulation and were not present in the control situation. All patients also showed some sort of differences in coherence intra- and/or inter-hemispheric in control situation, regardless that the tinnitus was uni- or bilateral. These differences varied depending on the stage of sleep considered and the range of bands analyzed.

During sound stimulation most of the changes of coherence occurred in the N2 sleep stage both if considered overall coherence as well as the different bands separately; followed by stage N3, being in REM sleep the least amount of changes. In stage N2 the spindles showed most of the changes, followed by bands theta, delta and alpha in descending order. In stages N3 and REM most of the changes appeared in the delta band, followed by theta and alpha bands (Table 2).

Changes in the N2 stage appeared both in the sense of the increase as the decrease of coherence for the delta, theta and alpha bands; however, the spindles changed reducing coherence most of the time. During sound stimulation in stage N3 coherence decreased for all studied bands (delta, theta and alpha) except in patient M.A. in whom the coherence increased in the delta band in the inter-hemispheric electrodes. When the stimulation occurred during REM sleep the coherence increased two-thirds of the time, decreasing in the remaining third, for all the bands studied (Table 2).

Fig. 3 shows changes of coherence in a patient with unilateral tinnitus, stimulated at the right side. In this example only in stage N2 changes appear with the sound stimulation. Intra-hemispheric overall coherence decreased in both hemispheres and spindles only decreased at the right. There are differences in the spindles coherence between the right and left hemispheres during the silence; these differences are not maintained during sound stimulation (top inset). Inter-hemispheric coherence only shows differences in the spindles between temporal and frontal areas during sound stimulation. The differences between controls at all sleep stages were kept during stimulation.

3.2.1. *Intra-hemispheric coherence*

Intra-hemispheric changes, comparing between periods of sound stimulation and silence, appeared mainly in the left hemisphere, regardless of the stimulated side (binaural, right or left; F3-T3, 65% vs. F4-T4, 35%).

3.2.2. *Inter-hemispheric coherence*

Temporal electrodes (T3-T4) showed more changes of coherence than frontal ones (F3-F4) in responses to sound stimulation (72% vs. 28%). Four patients without differences between frontal and temporal coherence during control showed differences when sound stimulated T.L., M.A., E.P., L.G. (Table 2). Most of the inter-hemispheric coherence changes appear in stage N2 (53%) followed by stage N3 (37%) and REM (10%). Taking into account the different bands, the delta band was the most changed in all sleep stages (67%), with predominance in temporal areas (47%); while theta and alpha bands only change the coherence in stages N2 and N3 in three patients.

Two patients with unilateral tinnitus, monaurally stimulated on the left, showed consistent decreased coherence in intra-spindle frequency and delta band in the temporal electrodes during the N2 stage, and also in the delta band in temporal (patient M.A.) and frontal electrodes (patient M.B.) during REM sleep. The only patient with tinnitus on the right showed significant coherence decrease of the spindles in temporal areas during stages N2 (Fig. 3, insert). However, some patients with bilateral tinnitus also showed inter-hemispheric coherence changes with the binaural sound stimulation (S.O., F. C., E.P., L.G., Table 2). In summary, changes in inter-hemispheric coherence differed depending on the stimulated side and the functional state of the brain, i.e., the sleep stage. We cannot rule out any other unknown factors.

4. Discussion

The main results found by the sound stimulation during sleep in patients with tinnitus were that the largest number of changes, considering both the power spectrum and wave's coherence, occurred in stages N2 and N3. The delta and theta bands were the most changed, with important changes also in coherence of spindles during N2. All changes were more frequent in temporal areas.

While we know that in the spontaneous sleep there is a progressive change throughout the night on the power of the waves, our results show specific changes and sometimes go in the opposite direction to those described as part of the processes of homeostasis sleep [16]. We cannot rule out, however, that the natural evolution of sleep is also influencing our results.

These results demonstrate, once again, the functional interactions between auditory sensory input and sleep, in this particular case in patients suffering from tinnitus. Tinnitus is considered a misperception that appears without an external sound and involves many levels of the brain activity [17,18]. Sound stimulation during sleep in subjects with

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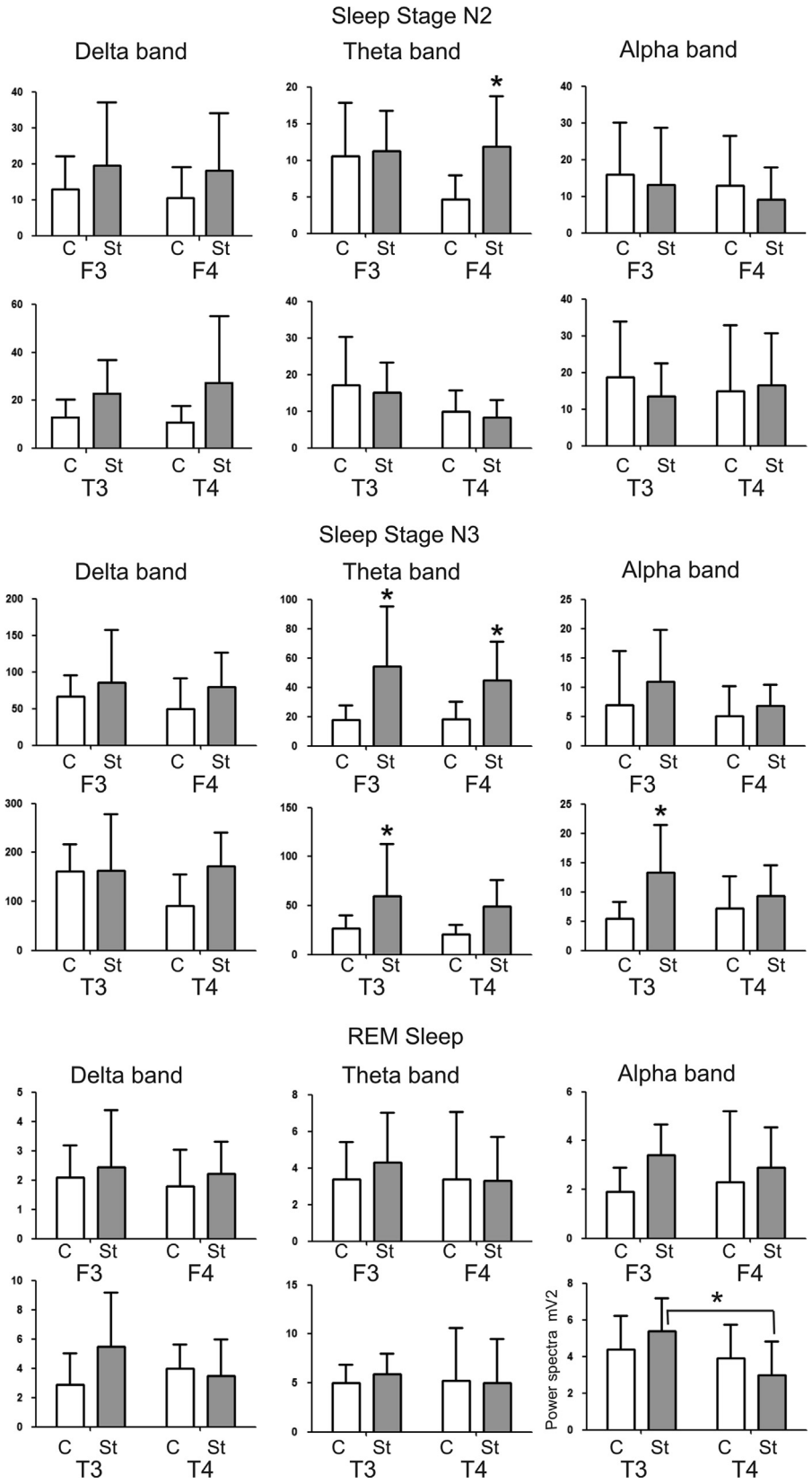

Fig. 2 – Power spectra of patient T.L. binaurally stimulated. Power spectra of delta, theta and alpha electroencephalographic bands were studied in frontal (F) and temporal (T) electrodes (3 is the left side, 4 is the right side), in each sleep stage (N2, N3 and REM). Ten temporal windows during sound stimulation (St) were compared with other 10 in silence as a Control (C) in each situation. Bars show means ± Standard Deviation. Data were statistically analyzed by one-way ANOVA followed by Tukey post hoc test to compare all pairs of columns, *$p < 0{,}05$.

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Table 2 – Coherence changes during sound stimulation in each sleep stage.

		Delta, theta, alpha, spindles		Overall coherence		
Patient	**Stage N2**	**Stage N3**	**REM**	**Stage N2**	**Stage N3**	**REM**
WF, m 42 y.o. *R.L*	Spindles F3T3 ↓	Delta F3T3 ≠ F4T4	Delta F3T3 ↑			
MB, f 64 y.o. *L*	Theta F4T4 ↑ Spindles F3T3 ↓ Spindles T3T4 ↓	Theta F3T3 ↓ Alpha F3T3 ↓	Delta F3F4 ↓		T3T4 ↓	T3T4 ↓
GB, f 39 y.o. *R,L*	Delta F3T3 ≠ F4T4	Delta F3T3 ↓ Theta F3T3 ↓ Alpha F3T3 ↓	Delta F3T3 ↑ Delta F4T4 ↑ Theta F3T3 ↑ Theta F4T4 ↑ Alpha F4T4 ↑		F3T3 ↓	
TL, f 32 y.o. *R,L*	Delta F3T3 ≠ F4T4 Spindles F3T3 ↓ Spindles F4T4 ↓	Delta F3T3 ≠ F4T4 Theta F3T3 ≠ F4T4 Theta F3F4 ≠ T3T4		F4T4 ↓		
SO, f 60 y.o. *R,L*	Delta F3T3 ≠ F4T4 Theta F4T4 ↑ Alpha F3T3 ↓ Delta T3T4 ↓ Alpha F3F4 ↑	Delta F3T3 ↓ Delta F4T4 ↓ Theta F3T3 ↓ Theta F4T4 ↓ Delta T3T4 ↓ Theta T3T4 ↓ AlphaT3T4 ↓	Theta F3T3 ↓	F3T3 ↓	T3T4 ↓	
FC, m 58 y.o. *R,L*	Theta F3T3 ≠ F4T4 Alpha F3T3 ↑ Spindles F3F4 ↑	Delta F3T3 ↓ Theta F3T3 ↓	Delta F4T4 ↑ Theta F3T3 ↑ Alpha F4T4 ↑			F3T3 ↑
MA, m 21 y.o. *L*	Delta F4T4 ↑ Theta F4T4 ↑ Theta F3T3 ≠ F4T4 Delta T3T4 ↓ Delta F3F4 ≠ T3T4 Alpha F3F4 ≠ T3T4 Spindles F3T3 ↓	Delta F3T3 ≠ F4T4 Alpha F3T3 ↓ Delta F3F4 ↑ Delta T3T4 ↑	Alpha F3T3 ↓ Delta T3T4 ↓ Delta F3F4 ≠ T3T4	F4T4 ↑ T3T4 ↓	F3T3 ↓	
EC, f 32 y.o. *R*	Theta F3T3 ↓ Theta F4T4 ↓ Spindles T3T4 ↓ Spindles F4T4 ↓			F4T4 ↓ F3T3 ↓		
EP, m 32 y.o. *R,L*	Alpha F3T3 ≠ F4T4 Delta T3T4 ↓ Theta T3T4 ↓ Alpha T3T4 ↓	Delta F3T3 ↓ Theta F3T3 ≠ F4T4 Delta F3F4 ≠ T3T4 Theta F3F4 ≠ T3T4 Alpha F3F4 ≠ T3T4	Delta F3F4 ≠ T3T4 Alpha F3F4 ≠ T3T4	T3T4 ↓		
LG, f 55 y.o. *R,L*	Delta F3F4 ↑	Delta T3T4 ↓ Delta F3F4 ≠ T3T4		F3T3 ↑	F3T3 ↑	

Table 2. Shows statistically significant coherence changes in each sleep stage (Stage N2; stage N3 and REM, rapid eye movements). Delta, theta and alpha bands intra-hemispheric coherence (F3-T3 and F4-T4) and inter-hemispheric coherence (F3-F4 and T3-T4) are compared during silent and sound stimulation. Also sleep spindles in stage N2 (13–16 Hz) were analyzed. ↑ and ↓, coherence increments and decrements during sound stimulation. ≠, when appeared differences between inter-hemispheric or intra-hemispheric coherences with stimulation, while they did not appeared in the "Control" situation. Only statistical significant changes are shown. Data were statistically analyzed by non-parametric ANOVA (Kruskal–Wallis test) and post hoc Dunn's Multiple Comparisons test.

tinnitus intended to induce changes in the neural networks, motivated by the fact that auditory processing continues during sleep [2,4] and the role of sleep in learning [5].

These results are consistent with previous studies supporting the idea that all sleep stages are necessary for information processing and learning during sleep [5]. The power of delta and

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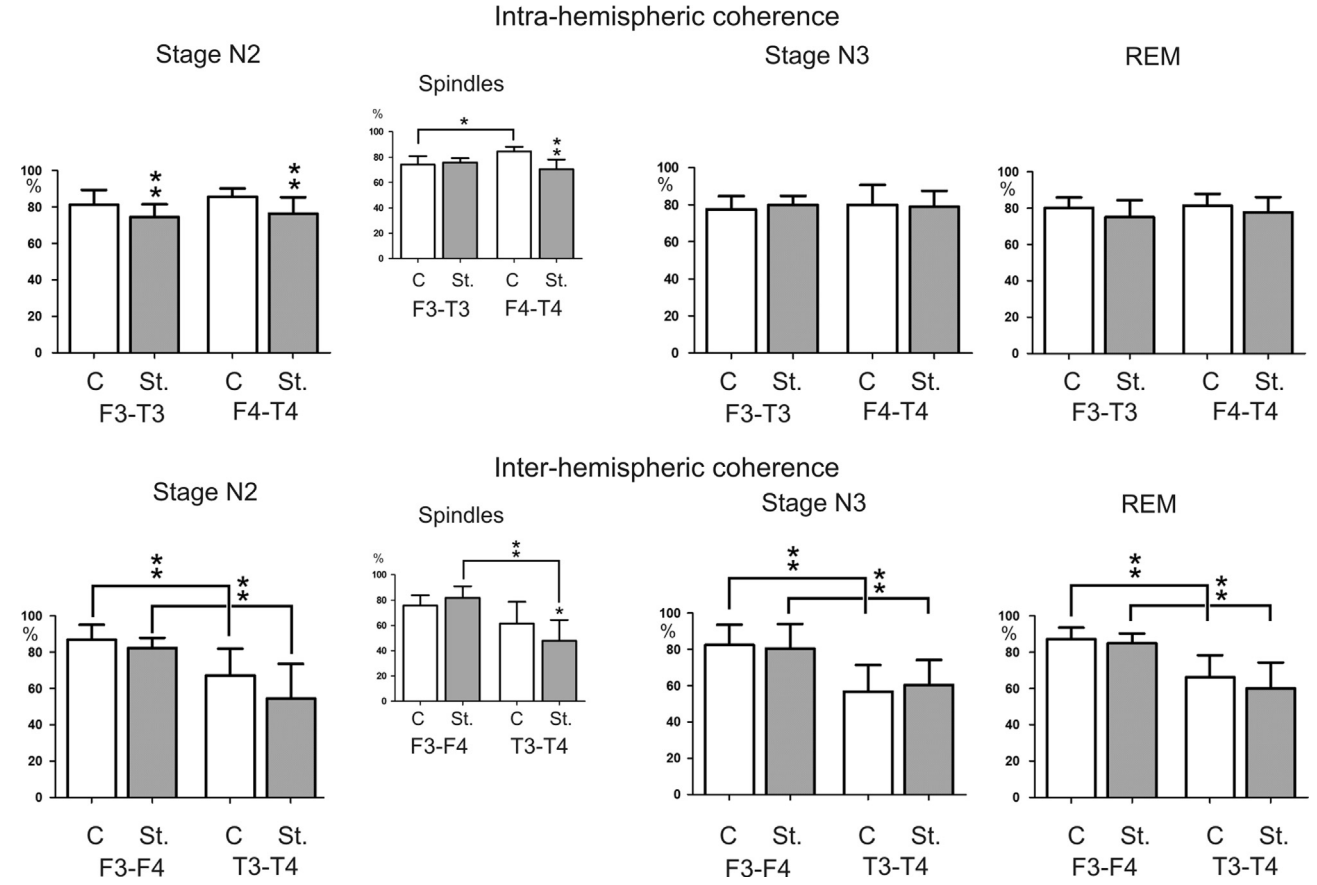

Fig. 3 – Overall coherence percentages in patient E.C. with monaural stimulation (right side). Overall coherence percentages were analyzed in intra-hemispheric electrodes (F3-T3 and F4-T4) and inter-hemispheric electrodes (F3-F4 and T3-T4) during stage N2, N3 and REM sleep. Insets, coherence of frequencies that make up the sleep spindles in stage N2 (13–16 Hz) were also studied. Bars show mean ± Standard Deviation. Data were statistically analyzed by non-parametric ANOVA (Kruskal–Wallis test) and post hoc Dunn's Multiple Comparisons test, $^{*}p < 0{,}05$, $^{**}p < 0{,}01$.

theta bands, prominent during sleep, increased with sound stimulation, thus strengthening their putative role. Previous studies of unitary activity in animal models have also strengthened the idea that delta and theta frequencies play an important role in sound processing during sleep [19–21]. Patients with fewer changes were those in which the tinnitus had disappeared or had decreased remarkably. However, the small number of patients does not allow speculate conclusions.

Inter-hemispheric differences in power spectrum appeared during both monaural and binaural stimulation, suggesting a dependence on factors other than the stimulated side, e.g., hemispheric dominance or other processes that are happening simultaneously. Even though the major changes in power spectra and coherence were observed in the temporal region, which is part of the auditory cortex, we also found changes in frontal regions. Changes in temporal and frontal regions have also been found in patients with intra-cochlear implants during sleep [22] and wakefulness [23]. Coexistence of frontal and temporal changes is not surprising since there is functional synergy between these areas.

In spite of new methods of quantitative analysis of electrical activity in the brain, the power spectrum remains the most used for providing information about the topographic distribution of energy in the EEG. Coherence techniques can be used to assess the functional relationship between cortical regions by quantifying the degree of cortical synchronization between areas within frequency bands [24]. Our coherence analysis showed that the brain changed during sound stimulation. We observed that increase or decrease in coherence depended on the frequency band and stage of sleep. However, the variability of the results indicates that unknown factors are also in play. Increased coherence has previously been reported during rhythmic auditory stimulation in both waking and slow wave sleep [25].

We found more change in intra-hemispheric coherence in the left than the right hemisphere. Since these shifts did not depend on the stimulated side we conclude that the differences are due to the different function of each hemisphere.

Inter-hemispheric coherence showed changes that do not depend, at least exclusively, on the stimulated side; being different the responses depending on the bands under consideration and the stage of sleep. Although patients with monaural stimulation showed inter-hemispheric differences, half of patients with binaural stimulation also presented changes, so we must assume that there will be many other

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unknown individual characteristics influencing the functional relationship between sensory processing and sleep.

It has been shown that intra-hemispheric coherence changes during the transition from wakefulness to sleep, being higher in the dominant hemisphere; the inter-hemispheric coherence asymmetry tends to disappear as the EEG progresses into sleep [26,27]. Cantero et al (1999) [28] also demonstrated that the same EEG activity can show different coherence pattern depending on the brain state. The lateralization for speech, music and pitch processing is widely accepted [29]. However, cortical responses are not known for sounds like those used in tinnitus treatment with customized sounds.

Our novel treatment was designed taking into account that sensory processing persists during sleep and that learning has been correlated with changes in EEG waves. The study is motivated by the hypothesis that a form of learning underlies the improvement observed in patients that undergo treatment. Our observation that sound stimulation induced changes in the same EEG waves that have been correlated with learning processes suggest that the treatment may be affecting neural networks involved in learning. Future studies should investigate inter-individual differences and the effect of sound stimulation in each sleep stage separately.

Acknowledgments

We are grateful to José L Peña for comments on the manuscript.

Partially supported by ANII (National Agency of Innovation and Research, Uruguay) and Cedar Sinai Medical Center, USA.

REFERENCES

[1] Velluti RA. Interactions between sleep and sensory physiology. A review. J Sleep Res 1997;6:61–77.
[2] Velluti RA. The auditory system in sleep. Amsterdam: Elsevier-Academic Press; 2008.
[3] Schaette R, König O, Hornig D, Gross M, Kempter R. Acoustic stimulation treatments against tinnitus could be most effective when tinnitus pitch is within the stimulated frequency range. Hear Res 2010;269:95–101.
[4] Pedemonte M, Drexler D, Rodio S, Geisinger D, Bianco A, Pol-Fernandes D, et al. Tinnitus treatment with sound stimulation during sleep. Int Tinnitus J 2010;16:37–43.
[5] Cipolli C. Sleep and memory. In: Parmeggiani PL, Velluti RA, Physiol Nat Sleep. London: Imperial College Press; 2005. p. 601–29.
[6] De Gennaro L, Ferrara M, Bertini M. The spontaneous Kcomplex during stage 2 sleep: is it the “forerunner” of delta waves? Neurosci Lett 2000;291:41–3.
[7] Mölle M, Born J. Slow oscillations orchestrating fast oscillations and memory consolidation. Prog Brain Res 2011;193:93–110.
[8] Mölle M, Bergmann TO, Marshall L, Born J. Fast and slow spindles during the sleep slow oscillation: disparate coalescence and engagement in memory processing. Sleep 2011;34:1411–21.
[9] Diekelmann S, Born J. The memory function of sleep. Nat Rev Neurosci 2010;11:114–26.
[10] Tononi G, Cirelli C. Sleep function and synaptic homeostasis. Sleep Med Rev 2006;10:49–62.
[11] Riedner BA, Hulse BK, Murphy MJ, Ferrarelli F, Tononi G. Temporal dynamics of cortical sources underlying spontaneous and peripherally evoked slow waves. Prog Brain Res 2011;193:201–18.
[12] Marshall L, Helgadottir H, Mölle M, Born J. Boosting slow oscillations during sleep potentiates memory. Nature 2006;444:610–3.
[13] Ngo H-V V, Claussen JC, Born J, Mölle M. Induction of slow oscillations by rhythmic acoustic stimulation. J Sleep Res 2013;22:22–31.
[14] Maquet PAA, Sterpenich V, Albouy G, Dang-bu T, Desseilles M, Boly M, et al. Brain imaging on passing to sleep. In: Parmeggiani PL, Velluti RA, Physiol Nat Sleep. London: Imperial College Press; 2005. p. 123–38.
[15] Portas C, Krakow K, Allen P, Joseph O, Armony JL, Frith CD. Auditory processing across the sleep–wake cycle: simultaneous EEG and fMRI monitoring in humans. Neuron 2000;28:991–9.
[16] Borbély AA, Achermann P. Sleep homeostasis and models of sleep regulation. In: Kryger MH, Roth T, Dement WC, Principles and Practice of Sleep Medicine, Fourth edition. Philadelphia: Elsevier-Saunders; 2005. p. 405–17.
[17] Jastreboff PJ. Phantom auditory perception (tinnitus): mechanisms of generation and perception. Neurosci Res 1990;8:221–54.
[18] De Ridder D. A heuristic pathophysiological model of tinnitus. In: Moller AR, Langguth B, DeRidder D, Kleinjung T, Textbook of Tinnitus. Springer; 2011. p. 171–97.
[19] Liberman T, Velluti RA, Pedemonte M. Temporal correlation between auditory neurons and the hippocampal theta rhythm induced by novel stimulations in awake guinea pigs. Brain Res 2009;1298:70–7.
[20] Pedemonte M, Pérez-Perera L, Peña JL, Velluti RA. Sleep and wakefulness auditory processing: cortical units vs. hippocampal theta rhythm. Sleep Res OnLine 2001;4(2):51–7.
[21] Velluti RA, Pedemonte M. Auditory neuronal networks in sleep and wakefulness. Int J Bifurcation Chaos 2010;20 (2):403–7.
[22] Velluti RA, Pedemonte M, Suárez H, Bentancor C, Rodriguez-Servetti Z. Auditory input modulates sleep: an intra-cochlear implanted human model. J Sleep Res 2010;19(4):585–90.
[23] Suárez H, Mut F, Lago G, Silveira A, de Bellis C, Velluti RA, et al. Changes in the cerebral blood flow in postlingual cochlear implant users. Acta Otolaryngol (Stockh) 1999;119(2):239–43.
[24] Cantero JL, Atienza M, Salas RM. Clinical Value of EEG coherence as electrophysiological index of cortico-cortical connections during sleep. Rev Neurol 2000;31(5):442–54.
[25] Başar E. Brain Function and oscillations. Integrative Brain Function. Neurophysiology and Cognitive Processes, Vol II. Berlin, Heidelberg, New York: Springer-Verlag; 1999.
[26] Boldyreva GN, Zhavoronkova LA. Interhemisphericasimetry of EEG coherence as a reflection of different functional states of the human brain. Biomed Sci 1991;2:266–70.
[27] Wada Y, Nanbu Y, Koshino Y, Shimada Y, Hashimoto T. Inter- and intrahemispheric EEG coherence during light drowsiness. Clin Electroenceph 1996;27:84–8.
[28] Cantero JL, Atienza M, Salas RM, Gómez CM, Alpha EEG. coherence in different brain states: an electrophysiological index of the arousal level in human subjects. Neurosci Lett 1999;271:167–70.
[29] Tervaniemi M, Hugdahl K. Lateralization of auditory-cortex functions. Brain Res Rev 2003;43:231–46.