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Music tuned to 432 Hz versus music tuned to 440 Hz for improving sleep in patients with spinal cord injuries: a double-blind cross-over pilot study

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Summary. *Background and aim of the work.* Among people with spinal cord injuries, sleep disorders are considered one of the top well-being priorities. Some studies suggest that listening to music promotes sleep and improves its quality. These studies usually used music standardised at the frequency of 440 Hz. The aim of the present study is to compare the effects of listening to music tuned to 440 Hz and music tuned to 432 Hz on sleep in patients with spinal cord injuries. *Methods.* Double-blind cross-over pilot study in a single Italian Spinal Unit. 12 patients with spinal injuries were provided with mp3 players loaded with their favourite music tuned to 440 Hz or 432 Hz. They were invited to listen to music for 30 minutes each day, in the two periods of the study. "Sleep Scale for Medical Study" modified for this study, and the "Perceived Stress Scale" were chosen. *Results.* The participants were eight males (mean age =58.12, SD ±13.62), and four females (mean age =56.25, SD ±14.17). Five were quadriplegics and seven were paraplegics. Listening times and wash-out periods were variable. The stress decreased, but not significantly, with listening to music at both frequencies. After listening to music at 432 Hz there was a significant improvement in sleep scores (+3.6, p=0.02), while there was no improvement in sleep scores listening to music at 440 Hz (-1.50, p=0.34). *Conclusions.* The results suggest that further studies on music interventions at 432 Hz should be performed. It is advisable to increase sample sizes and use a range of different research methods. (www.actabiomedica.it)

Keywords: Music, 432Hz, 440Hz, Sleep, Stress, Spinal injuries, Patients

Introduction

The use of music in clinical settings falls into two categories: music therapy and others music-based interventions (1). Music therapy is used to achieve individualised goals within a therapeutic relationship by an accredited professional who has completed an approved music therapy programme (2). Musical interventions, on the other hand, involve listening to recorded or live music to achieve general goals (e.g. to promote well-being, improve mood and achieve relaxation), and do not require the presence of a qualified professional (3). Musical interventions can be performed by operators who are not specialists in music and so can be offered as complementary activities to traditional therapies (3-4). Musical interventions, being inexpensive, easy to administer and without adverse effects, are already applied in several settomgs. For example, musical interventions improve social relationships and wellbeing (5) and contribute to the reduction of anxiety in hospital patients (6). In fact, musical interventions are implemented to reduce pre-operative anxiety (7-8) and

the perception of pain, lowering the need for analgesics (9). Moreover, musical interventions have shown a significant impact on reducing overall agitation of people with dementia in long-term care settings (10).

The results of a meta-analysis confirm that musical intervention is simple and safe, and suggest that it can improve sleep quality in different groups of people (11). Poor quality of sleep is linked to physical and mental health problems, with short-term negative effects being evident after just one night of poor sleep. Short-term memory can be compromised (12) and lower happiness levels and higher rates of depression are reported (13). If short-term sleep disturbance is not addressed, there is a risk of becoming chronic, with serious health and wellness problems (14-15).

Trahan et al. (16) provided insights into the relationship between music and sleep in a sample that varied widely in terms of age, sleep habits, stress levels and musical tastes.

Poor quality of sleep is a common problem in people with spinal lesions (17), and it is related to the kind of their injuries (18). Their devastating neurological condition can lead to various degrees of motor and/or sensitive deficits and paralysis, with loss of function and poor quality of life, and increased morbidity and mortality (19).

The sounds that make up music are generated by waveforms and their frequency is expressed in Hertz (Hz) (20). Frequency values influence the tone and timbre of musical sounds (21). The music used in the above studies had a frequency of 440 Hz, because this is the current reference frequency for tuning musical instruments (22-23). It corresponds to the LA3 (A4) musical note in the central octave of the piano (21). Tuning can also be done at 432 Hz. This is used sometimes in the New Age genre (for meditation music) (21) and by some musicians. The transposition of music from 440 Hz to 432 Hz can be easily achieved using musical editing softwares (24).

The benefits of tuning at 432 Hz have been discussed in some books (22, 25, 26, 27) and web publications (28-30). These sources suggest that listening to 440 Hz music can promote a feeling of anxiety, nervousness or aggression. In contrast, Di Nasso et al. (31) suggest that listening to music at 432 Hz can be useful to reduce anxiety and pain during dental care. But in this setting, there has been no comparison between the effects of listening to music at 432 Hz and music at 440 Hz.

Probably, the benefits of listening to music are not related to musical genre (e.g. classic, jazz, rock, pop music), but to the frequency and its tonalities.

However, to date, there is only one published pilot study that explored some specific effects of 440 Hz music compared to music at 432 Hz. The results suggested a positive impact of 432 Hz music on the change of vital signs, as a reduction in heart and respiratory rates (-4.79 beat per minute, p=0.05 and 1 breath per minute, p=0.06, respectively) (32).

Aim

The aim of this study was to detect the effects of listening to music at the frequency of 432 Hz on sleep, stress and other variables perceived (e.g. mood, appetite, energy) by subjects with spinal cord injuries and compare them to those obtained by listening to music at 440 Hz.

Patients with spinal lesions were selected because they usually undergo to many pharmacological and highly technological treatments (33), and there are few published studies exploring the effects of non-pharmacological interventions, such as listening to music, in improving their sleep quality.

Materials and Methods

Study design and participants

A pilot study with double-blind cross-over design was conducted.

A convenience sample was used for this study, with randomised assignment to the experimental group (listening to music at 432 Hz) and to the control group (listening to music at 440 Hz), reversing the groups in cross-over mode after a wash-out period.

The study included 12 patients staying in the Spinal Injuries Unit of Careggi University Hospital (Florence, Italy) between December 2018 and March 2019, aged over 18 years, with no reported or referred hearing problems or other hearing-related issues (hearing loss

or deafness, recent otolaryngologic interventions, otalgia), who wished to listen to music during the study's period and gave their informed and written consent.

Before starting the study, patients listened to music just occasionally, using their mobile phone, computer or television. None of the patients had an insomnia diagnosis documented in their individual medical notes.

Interventions and procedure

Each patient was provided with an mp3 player with earphones (Tabmart Metal Hi-Fi) and was provided with their chosen music. Musical tracks were either at 432 Hz or 440 Hz, depending on patients' randomised assignment to the experimental or control group in the first cross-over period. The tracks were converted for listening at 432 Hz (from the original frequency of 440 Hz) by a sound engineer, using the software Audacity© (34).

Patients were invited to listen to music stored on the mp3 players, at times of their choosing, for at least 30 consecutive minutes a day and for 10 continuous days. In this period, the patients were asked to listen only to the music contained in the assigned mp3 players. Listening was blinded.

Before the start of the listening period and at the end of the period, patients' quality of sleep and stress levels were measured with specific instruments. The data collections were done blindly.

During the period of the study, patients regularly attended all the ward's planned activities and therapies (eg. physiotherapy sessions) that didn't include listening to music.

At the end of the listening period patients were invited to notify any other factors arising in the listening period that could have interfered with their sleep or their stress levels (disturbing events, for example troubles relating to family members or problems with healthcare staff), affecting the results and the adherence to the required intervention. This information was collected by the researchers to be included in the results evaluation.

At the end of the first listening period the mp3 players were withdrawn and a wash-out period was implemented. Then the groups to which patients had been allocated during the first period (experimental and control) were reversed. The music in patients' mp3 players was converted to the opposite frequency (music at 432 Hz was converted to 440 Hz and conversely) and the mp3 players were returned to the patients. The second listening period was comparable to the first for number of days and daily time of music listening. During this second period, the same instruments of the first period, concerning sleep and stress, were administered.

Instruments

Four questionnaires were used for data collection.

Before starting the study, some socio-demographic and clinical variables were collected for each patient, favorite music (Questionnaire 1).

To estimate the quality of sleep, the "Sleep Scale from the Medical Outcomes Study" (Questionnaire 2) (35) was chosen. It contains 12 questions about the time needed to fall asleep, the number of hours of sleep and also about the quality of sleep (e.g. feeling of rest in the morning, snoring at night, naps during the day.).

For the present study the Sleep Scale from the Medical Outcomes Study was translated into Italian through translation and back-translation method. Moreover, it was modified because it was not considered adequate for the study. In particular, compared to the version used in the study by Muriel Viala-Danten et al. (36), the first two questions of Sleep Scale from the Medical Outcomes Study were estimated independently (minutes to fall asleep and average number of hours slept each night). The other 10 dimensions generated a total score ranging from 10 (bad sleep) to 50 (great sleep). The modified version of the instrument is shown in Table 1.

The scale was tested on five other healthy subjects using Cohen's kappa. A good match was obtained (K=0,8) and it was considered adequate for the study.

To estimate stress level, the Italian version of the "Perceived Stress Scale" (PSS), translated by University San Raffaele of Milan (available on the web), was used (Questionnaire 3) (37). The PSS includes 10 questions about nervousness and stress, feelings of trust, anger, a sense of control and loss of power in situations. The resulting variable was treated as a continuous variable ranging from 0 to 40, with lower values denoting lower stress levels. In the present study, the questions

featured in the PSS were referred to the days when music was listened to.

After the second listening period, a questionnaire was administered to the participants, to identify the degree of adherence to the study protocol, subjective perceptions of the changes that took place in the study period (with an open question), and any new factors that occurred during the observation period (Questionnaire 4). The administration times for the questionnaires are shown in Table 2.

Data analysis

The data collected were analyzed using the SPSS, version 17.0 statistical software.

The socio-demographic characteristics of the participants were reported with descriptive statistics, by calculating frequencies, percentages, range, averages and standard deviations where indicated.

Given the impossibility to set a fixed wash-out time for the interventions and the absence of reported standards timing from wash-out in literature of music intervention study, the first part of the analysis (6 subjects per group) was conducted using only the data

Table 1. Modified version of the Sleep Scale from Medical Study

INDEPENDENT ITEMS **(Are NOT included in the global score)**					
How long did it you usually take for you to fall asleep during the past 4 weeks? (Minutes to fall asleep).	0-15 minutes	16-30 minutes	31-45 minutes	46-60 minutes	>60 minutes
On the average, how many hours did you sleep each night during the past 4 weeks?	Average number of hours slept each night				
ITEMS THAT DEFINE THE GLOBAL SCORE **(Sleep total score 10- 50)**					
ANSWERS OPTIONS	**Always**	**Often**	**Sometimes**	**Rarely**	**Never**
SCORES	**1**	**2**	**3**	**4**	**5**
ITEMS	1) Feel that your sleep was not quiet (moving restlessly, feeling tense, speaking, etc., while sleeping)?				
	2) Get enough sleep to feel rested upon waking in the morning? *				
	3) Awaken short of breath or with a headache?				
	4) Feel drowsky ore sleepy during the day?				
	5) Have trouble falling asleep?				
	6) Awaken during your sleep time and have trouble falling asleep again?				
	7) Have trouble staying awake during the day?				
	8) Snore during your sleep?				
	9) Take naps (5 minutes or longer) during the day?				
	10) Get the amount of sleep you needed? *				

Note: In answering, participants were asked to refer to the previous days (which related to the listening period or to the wash out times). In the total score calculation, the scores for items 2 and 10, marked with an asterisk (*), were assigned in reverse (always=5; often=4; sometimes=3; rarely=2; never=1).

Table 2. Study design (N=12)

START	**First treatment_starts**		**Wash out**	**Second_reverse treatment**		**THE END**
After the enrollment of the subjects and before the randomizing of the treatments	**Time 0**	**Time 1**		**Time 0**	**Time 1**	**At the end of the study**
Questionnaire 1						Questionnaire 4
	Modified sleep questionnaire (Questionnaire 2)	Modified sleep questionnaire (Questionnaire 2)		Modified sleep questionnaire (Questionnaire 2)	Modified sleep questionnaire (Questionnaire 2)	
	PSS (Questionnaire 3)	PSS (Questionnaire 3)		PSS (Questionnaire 3)	PSS (Questionnaire 3)	

Legend: PSS - Perceived Stress Scale

from the first phase of the cross-over study. This choice was made to avoid any influence potentially exerted by the previous period of standardized listening.

After this, the data obtained from the first and second phases of the cross-over study were combined, to analyze their overall differences. The scores for each patient relating to stress and sleep quality before and after the experimental and control intervention were noted. The averages and the differences between the averages for each group of patients before and after the treatments were calculated.

After having verified the normality of the data distribution (Agostino's test), the authors ran the Student's T (Test, to analyze the difference between the average scores for sleep and stress for the time periods 0 and time 1 for both listening groups).

The p value ≤0.05 was considered significant.

Ethical Considerations

The study authorization was obtained from the Direction of Spinal-Injuries Unit of Careggi University Hospital. The authors also obtained the authorization of the local referring Ethics Committee for Clinical Experimentation. The number of ethical committee authorization procedure was 13628_spe. The number of study protocol authorization by the hospital management was ID 13628 –EM 24-2018.

The study respected the standards for information privacy based on art.13 of the Italian Legislative Decree n. 196 of 30 June 2003, and subsequent changes. The study was conducted according to the Good Clinical Practice (GCP) as set out in the last version recognized in Italian regulations, in the Helsinki Declaration, and according to other current and relevant regulations.

All the data collected were stored in a database that was accessible only to the researchers through password, thus protecting the confidentiality and the anonymity of the patients.

Results

Socio-demographic characteristics of the participants

The study involved 12 patients, of which 8 males with an average age of 58.12 (SD ± 13.62). 5 of them suffered from paraplegia and 3 from quadriplegia. 7 were hospitalized for less than 3 months.

The 4 females had an average age of 56.25 (SD ± 14.17), of which 2 suffering from quadriplegia and 2 from paraplegia. Only 1 of them had a length of stay in hospital less than 3 months.

The participants' favorite music genres were: Classic, International Pop, Italian pop, American country, Rock, Celtic, Meditation, Progressive and Reggae.

Table 3 reports the general characteristics of the participants from the Questionnaire 1.

Comparison between sleep and stress with music at 432 Hz and music at 440 Hz listened in the first period (6 patients per group).

Table 3. Characteristics of the particiapnts (N=12)

Variables	Male n 8	Female n 4
Age, average years (SD; range)	58.12 (±13.62; 30-54)	56.25 (±14.17; 43-69)
Time spent in hospital n (%)		
>3 months	1 (12.50%)	3 (75%)
<3 months	7 (87.50%)	1 (25%)
Reasons for admission (%)		
Polyrauma	6 (75%)	2 (50%)
Lumbar vertebral stenosis and multiple discopathies	1 (12.50%)	
Descending thoracic aorta aneurysm surgery	1 (12.50%)	
Surgically treated spinal infection		1 (25%)
Post-surgical epidural haematoma		1 (25%)
Outcomes of spinal cord injury n (%)		
Paraplegia	5 (62.50%)	2 (50%)
Tetraplegia	3 (37.50%)	2 (50%)
Main diseases present n (%)		
Cardiopathy	1 (12.50%)	1 (25%)
COPD	1 (12.50%)	1 (25%)
Diabetes mellitus type 2	3 (37.50%)	
Arterial hypertension	5 (62.50%)	
Dyslipidemia	2 (25%)	
Bipolarism		1 (25%)
Depressive syndrome		2 (50%)
Drug addiction		1 (25%)
Drug therapy n (%)		
Opiates	2 (25%)	2 (50%)
Muscle relaxants	1 (12.50%)	2 (50%)
Antidepressants	2 (25%)	3 (75%)
Anti-coagulants/anti-aggregants	7 (87.50%)	4 (100%)
Gastroprotectors	3 (37.50%)	2 (50%)
Antipsychotics		1 (25%)
Diuretics	3 (37.50%)	
Antihypertensives	3 (37.50%)	
Benzodiazepines (to facilitate sleep)	4 (50%)	2 (50%)

(Continued)

Table 3. (Continued)

Type of Physiotherapy n (%)		
Gym Activities	6 (75%)	4 (100%)
None	2 (25%)	
Favorite music/choice n (%)		
Classic		2 (50%)
International Pop	2 (25%)	2 (50%)
Italian pop	4 (50%)	3 (75%)
American country	1 (12.50%)	
Rock	3 (37.50%)	1 (25%)
Celtic	1 (12.50%)	
Meditation	1 (12.50%)	
Progressive	1 (12.50%)	
Reggae		1 (25%)
Return home Saturday and Sunday (Life Bridge) n (%)		
YES	2 (25%)	2 (50%)
NO	6 (75%)	2 (50%)

During the first period, 6 people listened to music at the frequency of 432 Hz and 6 at 440 Hz.

Table 4 describes the general characteristics of the participants based on their listening group and the specifics related to the first intervention period.

The number of listening days and the time period of the wash-out were very variable due to reason related to patients' individual needs and clinical condition. For both groups, some subjects reduced the minutes to fall asleep (Table 5).

No significant improvement was recorded on the average of hours slept each night and the PSS scores in both groups of participants. With music tuned to 432 Hz, the average of the sleep total scores significantly increases (+7; p=0.05), but not with music tuned to 440 Hz (-2.17; p=0.38).

For both T0 and T1, the Table 6 sets out the average hours slept every night by participants, the average of the total scores from the sleep questionnaire, and the average total score from the PSS.

Among 6 subjects who listened to music at 432 Hz, 3 reported improvements in mood and movement (open question in Questionnaire 4). The other 3 reported any kind of improvement.

Among 6 subjects who listened to music at 440 Hz, only 2 reported having perceived improvements in mood and movement. The other 4 did not report any sort of improvement.

Comparison between sleep and stress with music at 432 Hz and with music at 440 Hz on total group (12 patients per group).

Table 4. Characteristics of the 6 patients that listened to 432 Hz music and 6 patients that listened to 440 Hz music in the first period of the study

	1 listening_432 Hz		**1 listening_ 440 Hz**	
Variables	**Male n 5**	**Female n 1**	**Male n 3**	**Female n 3**
Age, average years (SD; range)	55.60 (±16.19; 30-73)	45	62.33 (±9.07; 54-72)	60 (±14,73; 43-69)
Time spent in hospital n (% partial)				
>3 months		1 (100%)	1 (33.33%)	2 (66.66%)
<3 months	5 (100%)		2 (66.66%)	1 (33.33%)
Reasons for admission n (% partial)				
Polytrauma	3 (60%)	1 (100%)	3 (100%)	1 (33.33%)
Descending thoracic aorta aneurysm surgery	1 (20%)			
Lumbar vertebral stenosis and multiple discopathies	1 (20%)			
Post-surgical epidural haematoma				
Surgically treated spinal infection				1 (33.33%)
				1 (33.33%)
Outcomes of spinal cord injury n (% partial)				
Paraplegia	3 (60%)	1 (100%)	2 (66.66%)	1 (33.33%)
Tetraplegia	2 (40%)		1 (33.33%)	2 (66.66%)
Principal pathologies present n (% partial)				
Cardiopathy				
COPD			1 (33.33%)	1 (33.33%)
Diabetes mellitus type 2	1 (20%)			1 (33.33%)
Arterial hypertension	1 (20%)		2 (66.66%)	
Dyslipidemia	2 (40%)		3 (100%)	
Bipolarism	1 (20%)		1 (33.33%)	
Depressive syndrome		1 (100%)		
Drug addiction				2 (66.66%)
				1 (33.33%)

(Continued)

Table 4. (Continued)

Drug therapy n (%)				
Opiates	1 (20%)		1 (33.33%)	2 (66.66%)
Muscle relaxants	1 (20%)			2 (66.66%)
Antidepressants	1 (20%)	1 (100%)	1 (33.33%)	2 (66.66%)
Anti-coagulants/anti-aggregants	4 (80%)	1 (100%)	3 (100%)	3 (100%)
Gastroprotectors	3 (60%)	1 (100%)		1 (33.33%)
Antipsychotics		1 (100%)		
Diuretics			3 (100%)	
Antihypertensives	2 (40%)		1 (33.33%)	
Benzodiazepines (to facilitate sleep)	2 (40%)	1 (100%)	2 (66.66%)	1 (33.33%)
Type of physiotherapy n (%)				
Gym activities	5 (100%)	1 (100%)	1 (33.33%)	3 (100%)
None			2 (66.66%)	
Favorite music/choice n (% partial)				
Classic				2 (66.66%)
International Pop	1 (20%)	1 (100%)	1 (33.33%)	1 (33.33%)
Italian pop	2 (40%)	1 (100%)	2 (66.66%)	2 (66.66%)
American country	1 (20%)			
Rock	2 (40%)		1 (33.33%)	1 (33.33%)
Celtic	1 (20%)			
Meditation	1 (20%)			
Progressive	1 (20%)			
Reggae				1 (33.33%)
Return home Saturday and Sunday n (% partial)				
YES	2 (40%)			2 (66.66%)
NO	3 (60%)	1 (100%)	3 (100%)	1 (33.33%)
Days of listening n (% partial)				
3		1 (100%)		
4			1 (33.33%)	
7				2 (66.66%)
10	1 (20%)		2 (66.66%)	1 (33.33%)
13				

(Continued)

Table 4. (Continued)

Days of wash out period n, (% partial)				
1				
3		1 (100%)		2 (66.66%)
4	4 (80%)		2 (66.66%)	1 (33.33%)
7	1 (20%)			
			1 (33.33%)	
Events that may have affected the effects under investigation n, (% partial)				
Fighting with nurses	1 (20%)			
Change in drug therapy			1 (33.33%)	1 (33.33%)

Table 5. Minutes to fall asleep at T0 and at T1. Music tuned to 432 Hz vs 440 Hz (6 patients per group) during first listening period

MINUTES TO FALL ASLEEP	First group listening to music tuned to 432 Hz Frequency		First group listening to music tuned to 440 Hz Frequency	
	T0	T1	T0	T1
>60	2	2	3	3
46–60	0	0	1	0
31–45	0	0	0	1
16–30	1	0	1	0
0–15	3	4	1	2
Total	6		6	

Table 6. Comparison of Sleep and Stress at T0 and T1 between starting group with music at 432 Hz and starting group with music at 440 Hz (6 patients per group) during first listening period

Times	Variables	432 Hz	440 Hz
T0	Average of the hours slept each night	7.33 (SD±0.81)	4.67 (SD±1.96)
	Average of the sleep total scores	36 (SD±7.92)	33 (SD±4.47)
	Average of the stress total scores (PSS)	14 (SD±11.36)	23.17 (SD±7.38)
T1	Average of the hours slept each night	7.17 (SD±0,98)	5 (SD±1.78)
	Average of the sleep total scores	43 (SD±6.35)	30.83 (SD±7.41)
	Average of the stress total scores (PSS)	9.67 (SD±7.06)	20.67 (SD±9.33)
T Test Differences T0-T1 (p value)	Average of the hours slept each night	-0.16 (p=0.61) ns	0.33 (p=0.17) ns
	Average of the sleep total scores	7 (p=0.05) *	-2.17 (p=0.38) ns
	Average of the stress total scores (PSS)	-4.33 (p=0.26) ns	-2.50 (p=0.49) ns

*Legend: *=p value<0.05; **=p value<0.01; ***=p value<0.001; ns = not significant*

The average hours slept each night by each participant, the average of the total score obtained from the sleep questionnaire and the average of the total score obtained from the stress questionnaire were calculated, considering both T0 and T1 for each variable (Table 7). For both groups, some subjects reduced the minutes to fall asleep. Table 8 describes the time patients took to fall asleep at T0 and T1 for both listening sessions.

No significant improvement was recorded on the average of the hours slept each night and on the PSS scores in both groups of participants. With music tuned to 432 Hz, the average of the sleep total scores significantly increased (+3.67; p=0.02), but not with music tuned to 440 Hz (-1.50; p=0.22).

Patients' compliance with the protocol and perceived effects on their health

During the study, music was listened daily by all participants. Patients reported a daily listening period ranging between 30 minutes and 5 hours. Their favorite moments for listening to music were during period of physical activity in the gym, during lunch and dinner or immediately after lunch, or in the evening after dinner.

Concerning the issue related to blindness in the present study, the participants expressed themselves on music at 432 Hz, defining it clearer (2 subjects), slower, deeper, and more intense (1 subject). No statements were made related to the listening to 440 Hz music.

Table 7. Comparison Sleep and Stress at T0 and T1 for total group with music at 432 Hz and at 440 Hz (12 patients)

Times	Variables	432 Hz	440 Hz
T0	Average of the hours slept each night	6.12 (SD±1.90)	5.79 (SD±2.34)
	Average of the sleep total scores	32.58 (SD±7.52)	34.83 (SD±5.35)
	Average of the stress total scores (PSS)	15.66 (SD±8.72)	19 (SD±10.09)
T1	Average of the hours slept each night	6.16 (SD±1.91)	6.12 (SD±1.78)
	Average of the sleep total scores	36.25 (SD±9.18)	33.33 (SD±6.11)
	Average of the stress total scores (PSS)	14.33 (SD±8.46)	15.92 (SD±8.68)
T Test Differences T0-T1 (p value)	Average of the hours slept each night	0.04 (p=0.99) ns	0.41 (p=0.24) ns
	Average of the sleep total scores	3.67 (p=0.02) *	-1.50 (p=0.34) ns
	Average of the stress total scores (PSS)	-1.33 (p=0.60) ns	-3.08 (p=0.22) ns

*Legend: *=p value<0.05; **=p value<0.01; ***=p value<0.001; ns = not significant*

Table 8. Minutes to fall asleep T0 and T1. Music tuned to 432 Hz vs 440 Hz (12 patients)

MINUTES TO FALL ASLEEP	Listening to music tuned to 432 Hz Frequency		Listening to music tuned to 440 Hz Frequency	
	T0	T1	T0	T1
>60	4	4	4	4
46–60	1	0	1	0
31–45	1	0	0	1
16–30	2	3	1	0
0–15	4	5	6	7
Total	12		12	

The subjects declared improvement to mood as a perceived effect on their health, (7 subjects with music tuned to 432 Hz, 3 subjects with music tuned to 440 Hz). An increase in appetite was perceived with music tuned to 432 Hz.

The Table 9 describes the perceptions reported by participants after the two listening periods with respect to the differences between music at 432 Hz and music at 440 Hz, and the effects on their own health.

Discussion

This study explored the effects of listening to 440 Hz or to 432 Hz tuned music on sleep and stress of patients with spinal cord injury. To date, in literature there are no similar studies comparing the different tuning of music on sleep and stress.

Although a literature review suggests that listening to music tuned to 440 Hz can have a positive impact on people's sleep (11), in this study those who listened to music tuned to 440 Hz did not improve their sleep. Indeed, in relation to the first six patients who listened to music at 440 Hz (without previously listening to music at the experimental frequency), no-significant deterioration of sleep quality and small improvements in stress levels were detected.

The study showed instead that the first six patients who listened to music at 432 Hz, when not contaminated by any previous period of listening, did report a significant improvement in the quality of their sleep. Their stress levels decreased too, but not significantly.

After listening to music at 432 Hz no significant difference was detected between the average hours slept between T0 and T1 for all 12 participants, while the increased sleep scores and the quality of sleep were statistically significant. The average stress scores increased from the medium-high level at T0 to a medium level at T1 (but this result was not significant).

After listening to music at 440 Hz, no significant improvement in the study variables were observed, for the 12 participants.

The patients enjoyed the musical intervention (for both frequencies): they fully adhered to the protocol and almost all extended the listening time beyond 30 minutes. However, they defined music tuned to 432 Hz as qualitatively different than music tuned to 440 Hz. In particular, the first was perceived as clearer, slower, deeper and more intense than the second.

Patients also expressed themselves more after listening to music tuned to 432 Hz, that was perceived by some participants to be good for their mood, increase appetite, have a positive impact on energy levels, and leave participants feeling more relaxed.

The studies included in the meta-analysis conducted by Jespersen et al. (11) have shown that patients chose a range of different musical styles (e.g. Classic, New Age, Jazz). Similarly, in the Trahan et al. survey the 403 participants who used music to sleep also mentioned 545 performers and several musical genres (16). From these results, it is difficult to know if particular kinds of music are more effective than others in facilitating sleep.

Table 9. Participants' perceptions of the differences between listening to music at 432 Hz and at 440 Hz and effects on their health

VARIABLES	MUSIC AT 432 HZ	MUSIC AT 440 HZ
Kind of music	**Frequency n.**	
Clearer music	2	
Slower music	1	
Deeper and more intense music	1	
Effect perceived on their health	**Frequency n.**	
Improvement to mood	7	3
Increase in appetite	3	
Increase in energy	3	2
Greater tranquility and reduced agitation	2	1

Even in the present study, patients' preferences for different kinds of music were heterogeneous, and this leads us to suppose that the observed effects are related to the listening frequency (432 Hz) and not to the musical genre that patients listened to.

To study this aspect in detail, it would probably be appropriate to use music originally composed and recorded at 432 Hz rather than music originally composed at 440 Hz and then converted to 432 Hz using a music software. This because music composed and recorded at 432 Hz would have the maximum harmonic spectrum of the frequency, which with conversion can be lost.

In general, more high quality randomized controlled studies are necessary to estimate the efficacy of listening to music to facilitate sleep and especially for the management of insomnia (11), particularly as none of our participants was diagnosed with insomnia. In addition to using instruments to measure the perceived quality of sleep, these pieces of research would probably need to use other instruments such as, direct observation and specific instrumental clinical exams.

Furthermore, musical interventions should be organized as recommended by specific guidelines (38), including a detailed report of the musical intervention, as we tried to do in the present study.

Limitations of the study

The current study has several limitations, including the use of convenience sampling, the low number of participants, recruited in a single center. There was no also third control group (no music listening) because of the difficulty of enrolling more patients. The variability in listening days and in wash-out periods it was a limit too. Furthermore, in relation to the variables for sleep and stress, the instruments used were not completely validated in Italian.

It would also be helpful have a greater degree of control over the space in which the study takes place and events taking place during the experimentation period, to deal with potentially confounding variables.

Finally, the choice in conducting the study was focused on the music preferred by the patients and also on the different musical frequencies, but it was not focused on the type of music (e.g. sad or happy) that would still affect sleep.

This study only lays the foundation for other studies on the effects of music frequencies on the sleep of patients with spinal cord injury.

Conclusions

The results of this pilot study suggest that listening to subjects' preferred music tuned to 432 Hz has positive effects on sleep quality compared to music at a frequency of 440 Hz in patients with spinal cord injuries.

Therefore, it seems useful to continue with this research agenda.

In the literature there is a lack of studies that compare the effects on sleep and stress (but also on human health in general), of listening to music tuned to 432 Hz versus 440 Hz. The implementation of rigorous studies on these musical frequencies is recommended.

The impossibility of controlling most of the variables that may have further influenced the effects on sleep and stress during the listening period, the variability in listening days, and the short wash-out periods suggest it is necessary to repeat the survey addressing the numerous limitations of the current study.

Based on participants' positive comments about the quality of music tuned to 432 Hz, some researchers could be interested to go in deep with investigations on this issue.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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