Research Article

Effect of Subliminal Auditory Stimulation on Components of Auditory Late Responses and Functional Magnetic Resonance Imaging Data in Adults with Normal Hearing

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Highlights

- Subliminal perception can occur when an individual is exposed to auditory stimuli
- Subliminal stimuli have long-term effects on the central nervous system structures

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ABSTRACT

Background and Aim: The use of subliminal stimulation for unconscious effects on the target population is of great importance. while several studies have generated proper visual and auditory stimuli for subliminal stimulation, no study was found on the long-term effects of it. Therefore, this study aims to investigate the long-term effects of auditory subliminal presentation on the central nervous system structures using fMRI and Auditory Late Responses (ALRs).

Methods: Participants were 26 students with a mean age of 24.03±2.32 years. There was four group in study. First, fMRI was done and ALRs were recorded for all of them. Then, music files containing words embedded in them was presented subliminally to participants in groups A and B for 10 days, group C received music file without any subliminal stimuli and group D was control group. It was repeated after 10 days.

Results: The subliminal stimuli had significant effects on the amplitudes of P1, N1, P2, and P3 waves (F3=25.03, 25.41, 39.11, and 14.60; p<0.001). Between-group comparison showed significant changes in groups A and B compared to groups C and D (p<0.05). The difference in the recorded potential mean values showed the highest change for recording electrodes in the prefrontal, frontal, and central regions and the lowest change in parietal and occipital regions. There was no significant change for a latency component.

Conclusion: Subliminal stimuli, presented appropriately and continuously, can leave long-term effects on the central nervous system structure causing extensive changes in the people's attitude to a certain subject.

Keywords: Subliminal perception; unconscious perception; unconsciousness; subconsciousness; subliminal auditory stimulus

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Introduction

Subliminal perception indicates the impact of visual, auditory, and tactile stimuli on people which are below their conscious threshold of perception and are not perceived consciously, but change their behavior, attitude, and other behavioral aspects [1, 2]. Their impact varies depending on the task, sensory channel, masking level, and top-down mechanism [3, 4]. Visual cortex can be activated by the stimuli without reaching awareness. Prime stimuli are usually used in the field of vision; the prime stimulus is presented for a short time and is separated from the main stimulus by forward and backward masking while the patient is unaware of the presence of the subliminal stimulus. The studies using functional Magnetic Resonance Imaging (fMRI) have shown that if the prime stimulus is similar to the main stimulus, the activity of some cortical areas will decrease, which is known as repetition suppression [1, 5, 6]. In the field of subliminal and auditory perception, speech stimuli are the most meaningful input signals to the human auditory system. Imaging methods such as fMRI used for understanding the Central Nervous System (CNS), can directly assess the activity of neural network in the brain during the presentation of subliminal stimuli. The use of these methods for determining active regions involved in speech perception has shown that presentation of subliminal stimuli results in reduced activity of the insula and left superior temporal gyrus. On the other hand, subliminal stimuli can have deep and long-term effects [7-9]. Continuous signal presentation to the brain can cause long-lasting neuroplasticity; however, no study has examined whether subliminal stimuli can also have similar long-term effects on the CNS.

So far, different methods have been introduced for making auditory subliminal stimuli in different studies. Some have attempted to reduce the intensity of speech and use white noise or competitive stimulus. Another method is the presentation of dichotic stimuli and asking the patients to pay attention to only one of the input channels. Time-series compression method has also been used in some studies [8, 9]. The mentioned methods have some disadvantages and none of them are perfect. Maybe due to the lack of a specific protocol for making auditory subliminal stimuli, there are fewer studies in this field. In the present study, we aimed to evaluate the long-term effect of sufficient subliminal stimulation (by both dichotic listening and intensity reduction methods) on the CNS and related components of neuroplasticity by using Auditory Late Responses (ALRs) and fMRI.

Methods

Participants

Twenty-six students (13 females(of Tehran University of Medical Sciences (Audiology Department, School of Rehabilitation Sciences) with a mean age of 24.03±2.32 years (ranged 18–25 years) participated in the study. They were first examined using the related checklists. All of them were speaking Farsi and right-handed, with normal hearing and no history of neurological diseases. One participant was excluded due to having otitis media during the study and three were excluded due to having high noise during preprocessing of fMRI data. The remaining participants were assigned to three experimental groups A (n=8), B (n=6), C (n=6) and one control group (group D, n=6). An informed consent form developed by the National Brain Mapping Lab was signed by all of them, and a psychiatrist evaluated their readiness to participate in the study.

Stimulus

The words baar and khaar, which are almost similar in terms of duration and repetition rate in Farsi, were used to generate subliminal stimuli. Then, a three-minute instrumental music was played. Dichotic listening techniques and a signal to noise ratio of –10 dB were used to present the words subliminally. Each word was embedded in the music file intermittently with a reduction in its intensity in the right and left channels (20 times in total) (Figure 1). Finally, three music files (baar, khaar, and control) were prepared. To ensure subliminal presentation of stimulus, all three music files were played for 10 participants before the study and they were asked to indicate the words during listening using a closed-ended question on a 4-point scale. None of them provided significant answers regarding the awareness and identification of the presented words.

Event-related potentials

The ALRs were recorded for all participants before and after the intervention. A 32-channel Electro Encephalography (EEG) device (g.tec medical engineering GMBH, Austria), the international 10-20 system for electrode placement, and a sampling rate of 512 Hz were used. The impedance of the electrodes was below 5 kΩ and the data quality of all electrodes was assessed before recording. A band-pass filter of 0.5–25 Hz and a time window of 100–800 ms were used in the preprocessing phase. The EEG data were referenced to the average of two A1 and A2 electrodes. Then, the following steps were performed using the EEGLAB toolbox of MATLAB software: a) visual inspection of the signals, b) removal of non-cerebral artifacts using independent component analysis, and c) averaging clean epochs to extract ALR. The task design was done using the Psychtoolbox of MATLAB software. During the test, four words of baar, khaar, khaab, and baal were presented. Each word was presented 300 times randomly at an Inter-Stimulus Interval (ISI) of 800–1200 ms and at an intensity of 70 dB SPL in a quiet room using two head-level loudspeakers positioned at a distance of one meter and at a 45° angle. To maintain the participants' attention, they were asked to sit in a chair comfortably and look at the a fixation point on the computer screen. They were instructed to press a key on the right side of the keyboard when they heard the word khaar and a key on the left side of the keyboard when they heard the word baar and ignore the other two words. Since in previous studies, the effectiveness of auditory training by long-latency response waves has well been observed and the origin of these waves is from the upper levels of the brain stem to the primary auditory cortex [10, 11], the latency and amplitude of P1, N1, P2, N2, and P3 waves were calculated and the TopoPlots of the words were created for all participants in four groups.

Functional magnetic resonance imaging

All participants received fMRI before and after the intervention. A 3.0-Tesla whole-body MRI system with a standard 20-channel head coil (Prisma, Siemens, Germany) was used. The fMRI was done in two steps. First, a high-resolution structural response was recorded (FOV: 256 mm, flip angle: 7˚, TI: 1100 ms, TE: 3.53 ms, TR: 1800 ms). The second step was word presentation. The task design was done using the Psyctoolbox of MATLAB software. In this task, the words baar, baal, khaar, and khaab were presented randomly at an ISI of 10 s using an headphone at the individual's most comfortable level. The participants were asked to press a button on the right side of the keyboard when they heard the word khaar and a button on the left side when they heard the word baar and keep their eyes closed (FOV: 192 mm, flip angle: 90˚, TE: 30 ms, TR: 3000 ms). Then, the data were preprocessed and the first-level analysis and group analysis were done in SPM software.

Subliminal intervention

After recording ALRs and fMRI, the subliminal intervention was presented. The participants in the group A received a music file containing the word baar as a subliminal stimulus. The group B received a music file containing the word khaar as a subliminal stimulus. The group C received a music file with no subliminal stimulus. The control group received no intervention. The participant listened to the music files at moderate intensity 5 times a day for 10 days through a headphone. After 10 days, the assessments (fMRI and ALR recording) were done again.

Data analysis

Data analysis was done in SPSS v.17 software. The Kolmogorov-Smirnov test was used for testing normality of data distribution. The amplitudes of P1, N1, P2, and P3 waves had a normal distribution while the amplitude of the N2 wave and latencies of P1, N1, P2, P3, and N2 had a non-normal distribution. Boxplots were used to detect outliers and remove them. Three-way analysis of variance (ANOVA) was used to analyze the data with a normal distribution and the Wilcoxon test was applied to analyze the data with a non-normal distribution. The SMP software and XJView tool of MATLAB software were used to analyze the fMRI data.

Results

Three-way ANOVA was used to evaluate the effect of the intervention in different groups and different stimuli. Among the variables, the amplitudes of P1, N1, P2, and P3 waves and theta power band met the n criteria. The mean and standard deviation of the amplitudes of waves and theta power band before and after the intervention are presented in Appendix 1. The results showed the significant effects of different subliminal stimuli on the amplitudes of P1, N1, P2, and P3 waves ($F(3,11)=25.03$, 25.41, 39.11, 14.60; $p<0.001$), but their effects on the theta power was not significant ($F(3,11)=1.57$, $p=0.197$). The post hoc least significant difference test was applied to evaluate the difference between the groups, whose results showed a significant difference in all variables in groups A and B compared to group C and D (control group) ($p<0.05$). Moreover, the results showed an overall reduction in the amplitudes of the waves or lower amplitude change for baar stimulus in group A and for khaar stimulus in groups B (Figure 2).

To evaluate the amplitude, change in different recording channels for groups A and B, the pre-intervention mean amplitude was subtracted from the post-intervention mean amplitude for each channel. The greatest change was seen in the prefrontal, frontal, and central lobes and the lowest change was observed in parietal and occipital lobes (Figure 3).

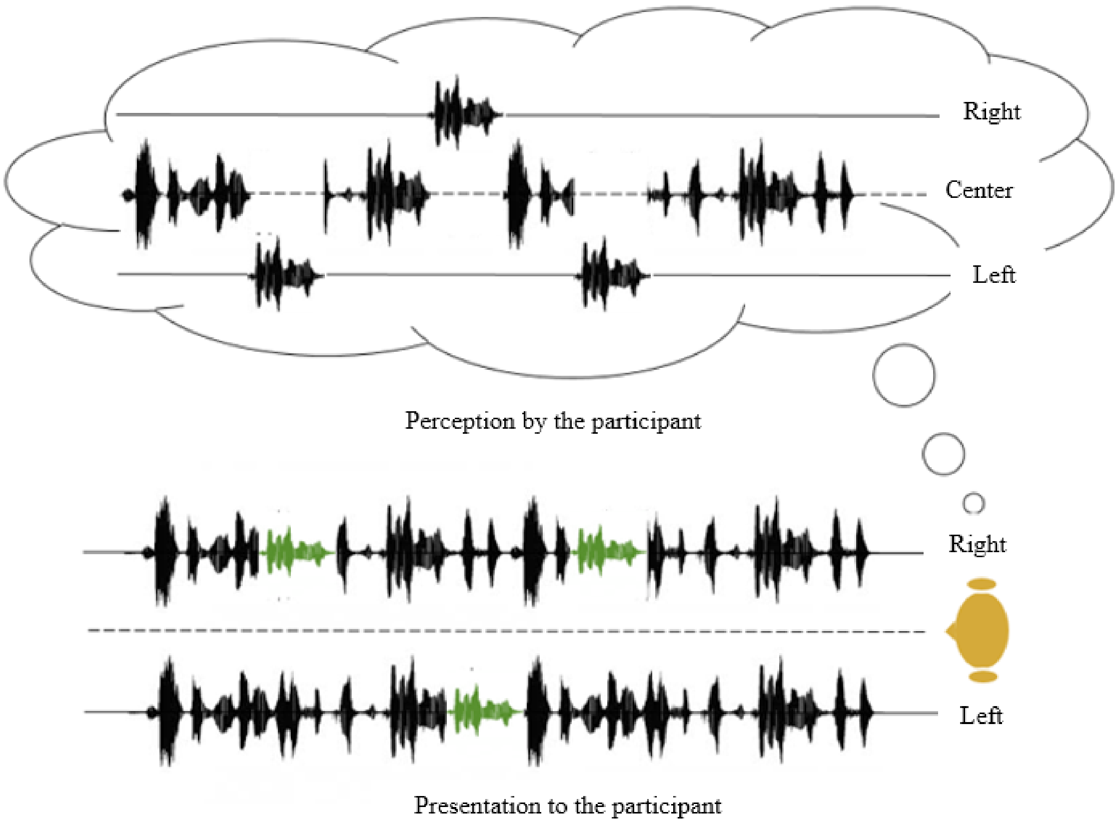

Figure 1. Presentation of subliminal stimuli

The Wilcoxon test was used to evaluate the data with a non-normal distribution, including the latency of all waves and the amplitude of N2 wave and alpha, beta, and delta power bands. The changes in these parameters before and after the intervention did not follow any significance patterns.

The SPM data were processed using the XJView tool of the MATLAB software. According to the voxel size,

Table 1. Activated cortical and subcortical regions before and after intervention

Before intervention		After intervention	
Region	**Activity level according to voxel size**	**Region**	**Activity level according to voxel size**
Frontal lobe	140	Frontal lobe	766
Superior temporal gyrus	126	Temporal lobe	735
Middle frontal gyrus	118	Superior temporal sulcus	553
Pre-motor cortex	117	Superior temporal gyrus	462
Superior frontal gyrus	112	Pons	360
Inferior frontal gyrus	104	Limbic system	355
Left cerebelum	98	Temporal planum	267
Cingulate gyrus	93	Thalamus	264
Thalamus	89	Cerebelum	252
Limbic system	81	Cingulate gyrus	236

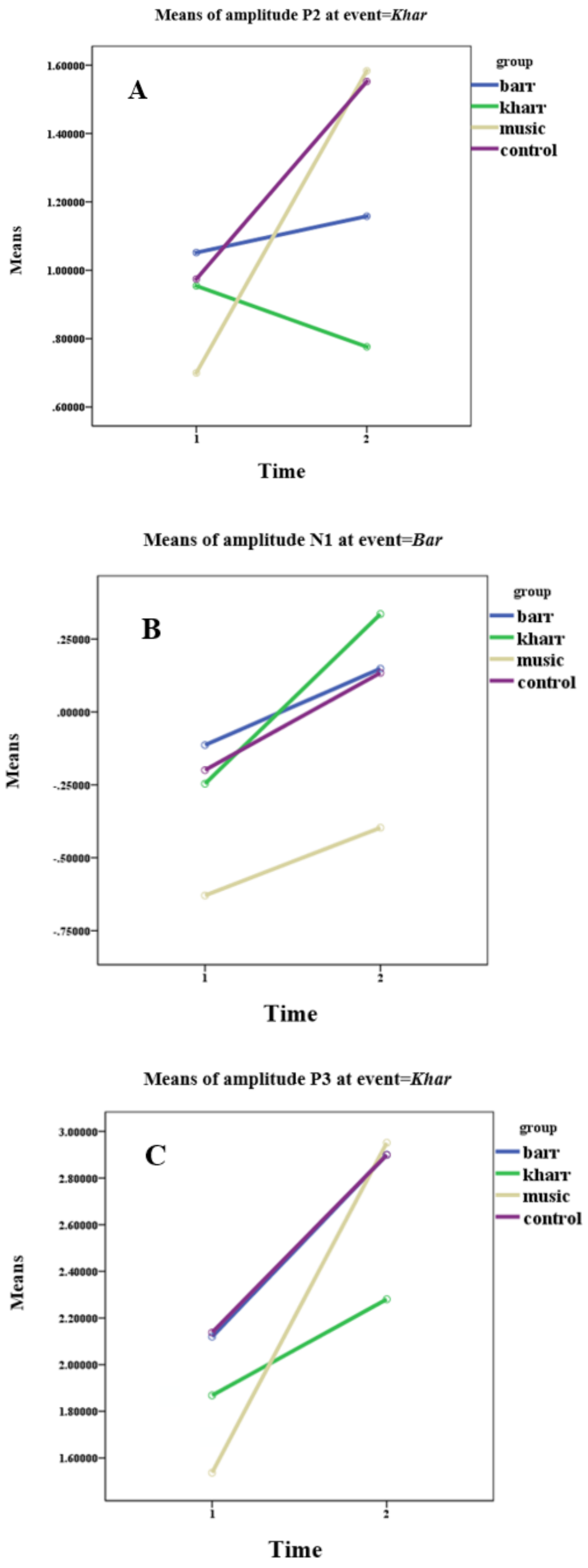

Figure 2. Changes in components of auditory late responses in different groups and conditions

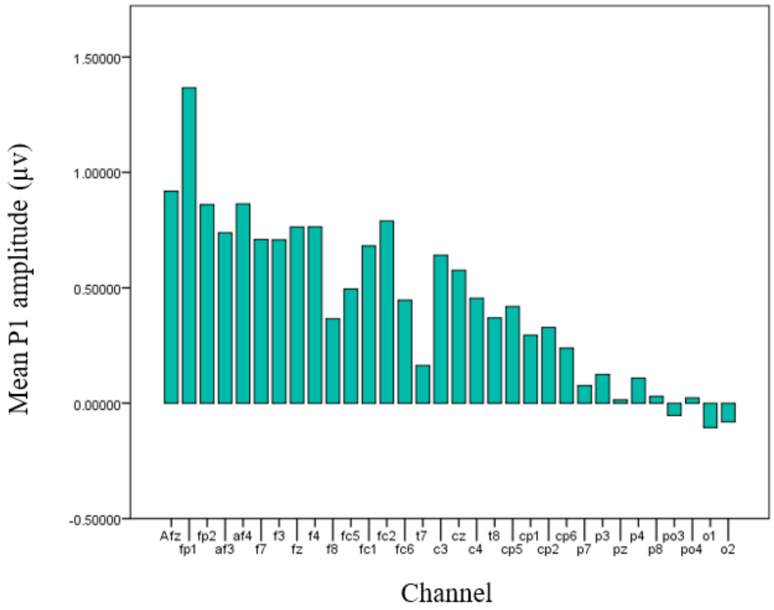

Figure 3. Mean P1 amplitude in different channels and regions using the event-related potentials recording method

the significant activity before the intervention were found in superior temporal gyrus, limbic system, middle, superior and inferior frontal gyrus, premotor cortex, thalamus, putamen, anterior cingulate, medial geniculate body, and medial and lateral cerebellum for both baar and khaar stimuli in A and B groups. (Table 1). After the intervention, the images showed increased activity of the superior temporal sulcus, superior temporal gyrus, planum temporale, and frontal lobe (Figure 4 and Figure 5).

Discussion

The present study was conducted to evaluate the long-term effects of auditory subliminal stimulation on different brain regions and the resulting neuroplasticity. For this purpose, ALR recording and fMRI were used to estimate the amount and extent of change in different brain regions. The results showed wave amplitude reduction using ALRs, or more specifically in ALRs. Several studies have shown that ALRs provide valuable information about speech decoding in the highest levels of the auditory cortex, which is due to more sensitivity of these areas to speech stimuli compared to simpler waves such as tonal waves. For example, it has been reported that the amplitudes of the P2 and N1 waves of ALRs are larger for speech stimuli compared to tonal stimuli [12, 13]. Our findings are consistent with the results of

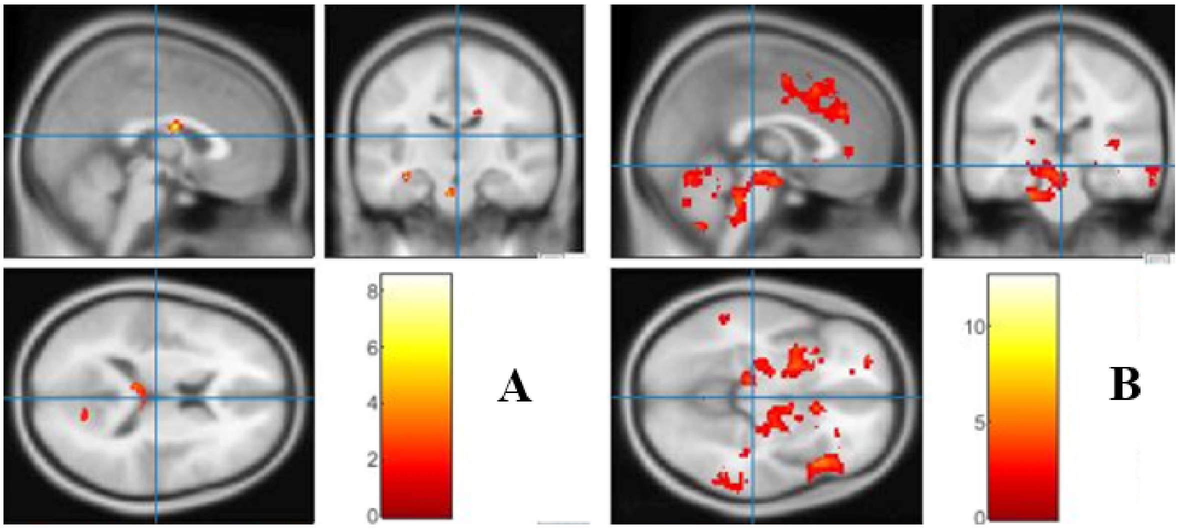

Figure 4. Changes in activity of the central nervous system regions before (A) and after (B) intervention (for group B and word /khaar/)

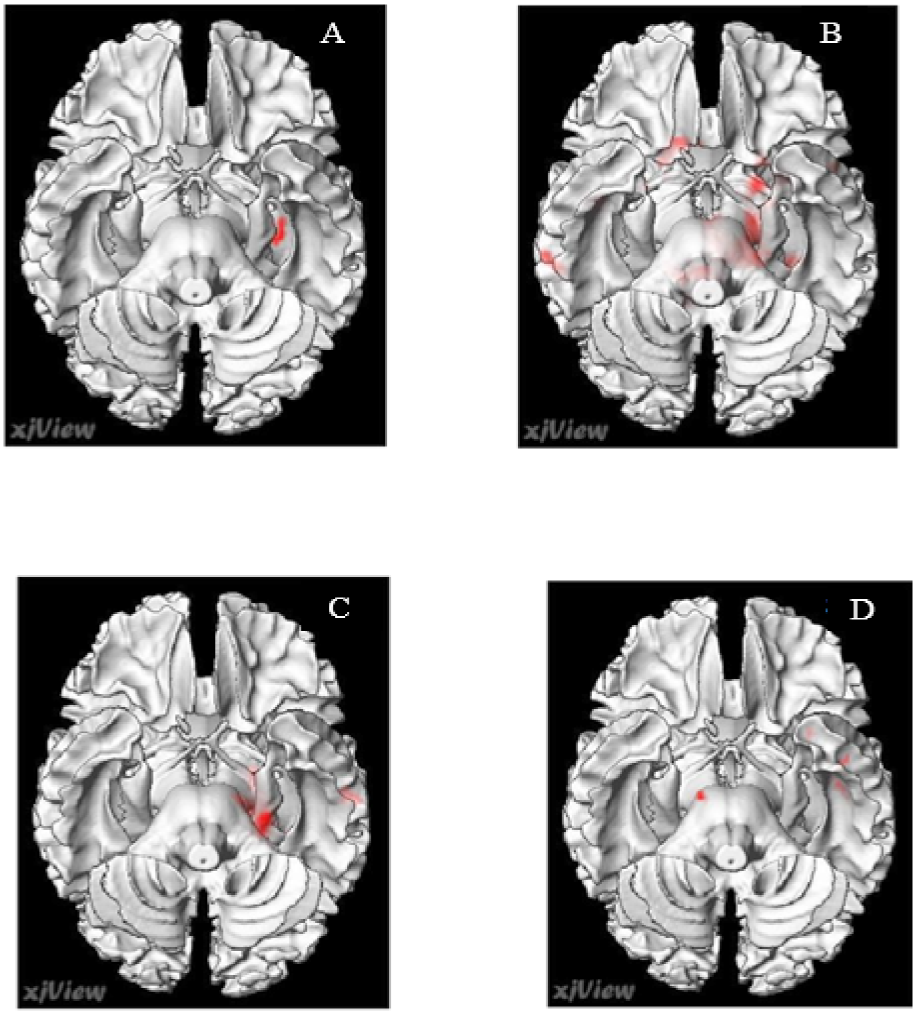

Figure 5. Central nervous system activity before (panels A and C) and after (panels B and D) of intervention for group A; subliminal stimulus baar (panels A and B), subliminal stimulus khaar (panels C and D)

previous studies that evaluated the effect of auditory training in brain structures using ALRs. Cunninghum et al. examined the effect of cue enhancement on the behavioral speech function and electrophysiological measurements in children with learning problems and their normal counterparts. They used frequency following responses and ALRs. They suggested that ALRs can be used to show the effectiveness of auditory training in the brain structures [14]. In Tremblay et al.'s two studies, it was shown that changes resulted from auditory training could be documented using P1, N1, and P2 components of ALRs. In their studies, the participants were not able to discriminate two separate sounds and perceived them as a single sound before auditory training. After training, the subjects could discriminate sounds. The effect of auditory training was evaluated using ALRs and the electrodes were placed in Fz, Cz, and Pz areas and in the right and left temporal and frontal lobes. Their results showed a reduction in the P1 amplitude and an increase in the N1 and P2 amplitude, which were noticeable for temporal and frontal electrodes. The authors concluded that ALRs can be used to show the effectiveness of auditory training [10, 11]. Bidelman et al. evaluated the effect of music therapy using ALRs and reported the effectiveness of intervention [15].

Regarding the long-term effects of subliminal stimulation using fMRI, our results showed that, in all groups, the activated brain regions and subcortical areas were the frontal lobe, superior temporal gyrus, middle frontal gyrus, premotor cortex, superior frontal gyrus, inferior frontal gyrus, left cerebellum, cingulate gyrus, thalamus, and limbic system. On the other hand, in cases where the presented and target stimuli were similar to subliminal stimuli, it was resulted in the activation of superior temporal sulcus and gyrus, planum temporale and frontal lobe in addition to above regions. Several studies have evaluated the activated brain areas using fMRI following the presentation of speech stimuli in passive or active modes. During passive listening, compared to the resting state as a control state, the activity of superior temporal gyrus (Heschl's gyrus and planum temporale) and superior temporal sulcus have been observed. These regions are related to the Wernicke's speech area and the prelinguistic preprocessing stage of hearing [9]. Similar to the function of the superior temporal gyrus in monkeys, this region in humans is responsible for coding many complex auditory information. Therefore, many differences between word perception at passive and resting-state modes are related to auditory perception processes and they cannot be attributed to word recognition. Hence, the brain regions that are activated when receiving spoken non-words and complex words (that are not speech words) should be similar, which has reported in several studies [16-18].

Semantic decision-making (active listening to words) requires attention to words, making decisions, and creating a motor response. Similar to inactive listening, this condition results in the bilateral activation of the superior temporal gyrus (stronger compared to inactive listening), lateral frontal gyrus, inferior frontal gyrus, middle frontal gyrus, premotor cortex, cingulate gyrus, insula, limbic system, cerebellum, and subcortical structures [19-21]. Some activities that are seen in active listening but not in passive listening can be due to general brain responses regardless of its specificity. For example, decision-making about a certain stimulus can result in activation of the attention system. In addition, any decision-making leads to activation of working memory for recalling instructions and having certain responses [22-24]. In overall, objective methods such as ALR recording and fMRI can indicate the effects of subliminal stimuli on the CNS structures. Longer duration of subliminal stimuli presentation and follow-up of patients are recommended in future studies to confirm long-term effects of subliminal stimuli.

Conclusion

Subliminal stimuli, presented appropriately and continuously, can leave long-term effects on the central nervous system structure causing extensive changes in the people's attitude to a certain subject.

Ethical Considerations

Compliance with ethical guidelines

Ethical approval was received from the Ethics Committee of Tehran University of Medical Sciences (Approval ID: IR.TUMS.FNM.REC.1399.023).

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Authors' contributions

SA: Study design, data collection and interpretation, writing the manuscript; GM: Study design and supervision on research; SF: Study design and supervision on research; SJ: Data analyzing; AP: Study design counseling; KPY: Data collection.

Conflict of interest

The authors declare that there is no conflict of interest.

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Appendix 1. Mean and standard deviation of auditory late responses in different groups and stimuli before and after of presenting subliminal stimuli

	Time	Stimuli	Group	Mean(SD)	N
P1 amplitude	Before	Bar	A	0.67(0.82)	222
			B	0.04(0.82)	190
			C	0.39(0.56)	190
			D	0.24(0.52)	122
		Khar	A	0.56(0.77)	224
			B	0.54(0.68)	192
			C	0.01(0.68)	190
			D	0.63(0.82)	128
	After	Bar	A	0.78(0.90)	222
			B	0.82(0.70)	190
			C	0.39(0.93)	190
			D	0.56(0.57)	122
		Khar	A	−0.03(0.85)	224
			B	0.47(0.84)	192
			C	0.45(0.89)	190
			D	0.82(0.63)	128
N1 amplitude	Before	Bar	A	−0.11(0.78)	222
			B	−0.37(0.87)	190
			C	−1.08(0.81)	190
			D	−0.08(0.60)	122
		Khar	A	−0.01(0.98)	224
			B	0.00(0.89)	192
			C	−0.90(0.70)	190
			D	−0.04(0.68)	128
	After	Bar	A	0.22(0.94)	222
			B	0.13(0.84)	190
			C	−0.26(1.05)	190
			D	0.14(0.52)	122
		Khar	A	−0.56(0.86)	224
			B	−0.06(0.74)	192
			C	0.03(0.98)	190
			D	0.22(0.64)	128

	Time	Stimuli	Group	Mean(SD)	N
P2 amplitude	Before	Bar	A	0.93(0.74)	222
			B	0.84(0.61)	190
			C	1.19(0.59)	190
			D	1.00(0.84)	122
		Khar	A	1.07(1.03)	224
			B	0.87(0.79)	192
			C	0.55(0.60)	190
			D	0.97(0.65)	128
	After	Bar	A	1.16(1.09)	222
			B	1.60(1.08)	190
			C	1.28(1.14)	190
			D	1.44(1.04)	122
		Khar	A	1.20(1.20)	224
			B	0.74(0.71)	192
			C	1.71(0.78)	190
			D	1.55(0.94)	128
P3 amplitude	Before	Bar	A	2.79(2.00)	222
			B	2.47(1.87)	190
			C	3.24 (2.58)	190
			D	2.72(2.55)	122
		Khar	A	2.16(1.66)	224
			B	1.75(1.16)	192
			C	1.90(1.47)	190
			D	2.13(1.69)	128
	After	Bar	A	2.60(2.02)	222
			B	3.06(2.92)	190
			C	4.10(2.64)	190
			D	2.35(1.95)	122
		Khar	A	2.94(1.80)	224
			B	1.96(1.64)	192
			C	3.95(2.60)	190
			D	2.89(2.04)	128

	Time	Stimuli	Group	Mean(SD)	N
Power Theta	Before	Bar	A	0.26(0.19)	222
			B	0.21(0.17)	190
			C	0.20(0.14)	190
			D	0.21(0.19)	122
		Khar	A	0.13(0.10)	224
			B	0.11(0.07)	192
			C	0.11(0.09)	190
			D	0.18(0.16)	128
	After	Bar	A	0.26(0.23)	222
			B	0.21(0.16)	190
			C	0.35(0.26)	190
			D	0.17(0.15)	122
		Khar	A	0.12(0.10)	224
			B	0.09(0.06)	192
			C	0.17(0.15)	190
			D	0.15(0.15)	128

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