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Brain responses to 40-Hz binaural beat and effects on emotion and memory

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ABSTRACT

Gamma oscillation plays a role in binding process or sensory integration, a process by which several brain areas beside primary cortex are activated for higher perception of the received stimulus. Beta oscillation is also involved in interpreting received stimulus and occurs following gamma oscillation, and this process is known as gamma-to-beta transition, a process for neglecting unnecessary stimuli in surrounding environment. Gamma oscillation also associates with cognitive functions, memory and emotion. Therefore, modulation of the brain activity can lead to manipulation of cognitive functions. The stimulus used in this study was 40-Hz binaural beat because binaural beat induces frequency following response. This study aimed to investigate the neural oscillation responding to the 40-Hz binaural beat and to evaluate working memory function and emotional states after listening to that stimulus. Two experiments were developed based on the study aims. In the first experiment, electroencephalograms were recorded while participants listened to the stimulus for 30 min. The results suggested that frontal, temporal, and central regions were activated within 15 min. In the second experiment, word list recall task was conducted before and after listening to the stimulus for 20 min. The results showed that, after listening, the recalled words were increase in the working memory portion of the list. Brunel Mood Scale, a questionnaire to evaluate emotional states, revealed changes in emotional states after listening to the stimulus. The emotional results suggested that these changes were consistent with the induced neural oscillations.

1. Introduction

Gamma oscillation is neural oscillation that maintain long-range communication in the brain (Fries, 2005) including the communication between thalamus and cerebral cortex, and within cerebral cortices themselves (Llinas et al., 2002). Thalamus and cerebral cortex, normally, communicate via thalamocortical circuit including 2 types of thalamic neurons which are thalamic core neurons, and thalamic matrix cells. The former sends projection fibers to specific cortical areas, i.e., primary auditory cortex while the later send projection fibers to widespread cortical areas, i.e., other auditory areas. These connections including connections within cerebral cortices communicate to one another by synchronization of neural activities which oscillate at the same frequency (Jones, 2009). The occurred oscillation which is generated for schema sending is gamma oscillation (Llinas and Ribary, 1993).

Gamma oscillation is associated with arousal maintenance of the brain during waking state (Vanderwolf, 2000; Gray, 1999; Gray and Singer, 1989; Llinas and Pare, 1991). It is found during consciousness and the oscillation pattern is interrupted when lack of consciousness occurs. These are clearly observed in rats. During normal postures, which are head held up against gravity, and eyes open, gamma oscillation pattern was found. After the rats were treated with some medicine to let them in stupor, gamma oscillation pattern was disrupted by suppression. However, the experiment indicated that slow wave oscillation was generated in the rats' brain when they were immobile in waking state. This slow wave is explained as similar to alpha oscillation in humans. Therefore, it is related to consciousness during waking state.

In addition to association to arousal and consciousness, gamma oscillation also plays an important role in sensory integration process (Ross and Fujioka, 2016; Llinas and Ribary, 1993; Mima et al., 2000; Pantev et al., 1991; Tallon-Baudry et al., 1996; Bertrand and Tallon-Baudry, 2000). When a stimulus is received by receptors and transduced into signal sending to the brain – primary area of the brain related to the signal is active to process the signal. This mechanism leads to sense that stimulus. However, to perceive higher perception or to process more detail of the stimulus, other brain areas related to the primary area must co-operate during the process (Fries, 2005; Ross and Fujioka, 2016; Gray et al., 1989). For example; after auditory information is received, primary auditory cortex analyzes that auditory stimulus (Harris et al., 2009). Other auditory features such as the meaning of that stimulus are required other brain areas to process (Harris et al., 2009; Vermeire et al., 2016). The process that integrates, groups, and analyzes the received information is called binding process

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or sensory integration. One piece of evidence supporting binding process is aging. Elderly are frequently found to be able to hear sound but unable to understand what has been heard (Martin and Jerger, 2005). This occurs because binding process is impaired in elderly (Ross and Fujioka, 2016; Vermeire et al., 2016). Binding process occurs by co-operation among several brain areas via connections between thalamus and cerebral cortex, and within cerebral cortices, themselves; therefore, that co-operation is synchronization among those brain areas. The mentioned synchronization is neural synchronization which corresponds to gamma oscillation. Therefore, gamma oscillation is an important mechanism underlying the binding process.

Another interested point is that gamma oscillation plays an important underlying role in cognitive functions (Fries, 2009; Fries et al., 2007; Ward, 2003; Jensen et al., 2007; Herrmann et al., 2004). By which attention level of an individual increases while gazing on an object, gamma oscillation also increases. However, when visual field of attention is shifted to the other side, gamma oscillation evokes in contralateral hemisphere (Ward et al., 2006). Another piece of evidence is gazing at a moving bar (Muller et al., 1996). In the previous study, there were 2 types of moving bars; the first type was one long bar moving in one direction, and the other type was two short bars moving in opposite direction. The first case, long bar, induced gamma oscillation in humans while the second case, 2 short bars, did not induce gamma oscillation. The results of both attention and moving bar support sensory integration or binding process. When attention level increases, several brain areas have to function to analyze higher perception of the stimulus. In addition, gazing at long moving bar requires 2 eyes coordinating together and leading to binding process while gazing at 2 short moving bars does not require coordination. A previous study claimed that gamma oscillation and memory process associates and links to each other (Tallon-Baudry et al., 1998). In that experiment, visual task was conducted in participants; when the same previous object was presented, gamma oscillation was observed in the brain at frontal sites. Moreover, prefrontal cortex is an important role in emotional responses beside amygdala, hippocampus, and anterior cingulate. Changes in emotional states induce gamma oscillation in prefrontal part of the brain (Damasio, 1995; Davidson, 1992; Davidson et al., 1990; Derryberry and Tucker, 1992; Davidson and Hugdahl, 1995). It can be clearly seen that gamma oscillation associates with cognition process.

Cognition is the mental action or process of acquiring knowledge and understanding through thought, experience, and the senses defined by oxford dictionary (English Oxford Living Dictionaries, n.d.). It includes several processes and mechanisms; for example, attention, memory and working memory, judgment and evaluation, reasoning and computation, problem solving and decision making, comprehension and language, and perception. Cognition in humans occurs both during consciousness and unconsciousness. Cognition, normally, can be changed along lifetime by surrounding environment, and new knowledge is also developed by existing knowledge; however, this concept seems to be abstract. Enormous studies have shown that gamma oscillation has been linked with cognition regarding several processes as mentioned.

The mentioned gamma oscillation is referred to 40-Hz oscillation (Nadasdy, 2010; Fries, 2009; Ross and Fujioka, 2016; John, 2002; Plourde et al., 1998). Gamma oscillation is generally displayed in frequency range of 30 to 100 Hz; however, 40-Hz is typically considered as gamma oscillation. This oscillation, 40-Hz, is generated by neural synchronization according to existing evidences. Firstly, 40-Hz auditory steady-state response (ASSR) expresses similar characteristics to fast-spiking interneurons which are hypothesized as main source of gamma oscillation (Vierling-Claassen et al., 2010). Secondly, combination of sensory inputs indicates inconsistencies with temporal dynamics of 40-Hz ASSR onset transition (Ross et al., 2002). This leads to another additional gamma oscillation components. Lastly, changing of stimulus feature or changing of stimulus resets 40-Hz oscillation (Ross, 2008; Ross and Pantev, 2004; Ross et al., 2005), as explained by the binding process. These findings support that 40-Hz oscillation represents gamma oscillation of interest based on several aspects mentioned previously: long-range communication of the brain, sensory integration or binding process, and cognitive related functions.

Another interesting issue of gamma oscillation is the change from gamma oscillation to beta oscillation which appears after a sufficiently high stimulus intensity is received (Traub et al., 1999; Haenschel et al., 2000). This changing phenomenon is called gamma-to-beta transition. As a stimulus is received into the brain, gamma oscillation occurs in response to that stimulus; if the intensity of the stimulus is high enough, the beta oscillation subsequently appears as a salient activity. Gamma oscillation is normally generated in primary sensory cortex in response to the stimulus and other specific association areas (Barth and MacDonald, 1996; Pantev et al., 1991; Pantev, 1995); however, occurred beta oscillation induced by the stimulus is generated in association areas and widespread areas across the cerebral cortex (Roelfsema et al., 1997; von Stein et al., 1999). In addition, prefrontal cortices also express the beta oscillation (Giard et al., 1994; Loveless et al., 1996; Lu et al., 1992). This gamma-to-beta transition is seemingly explained as sensory gating (Clementz et al., 2002; Crawford et al., 2002; Hong et al., 2004) which is neural process to filter out unnecessary stimuli in the brain from several stimuli in surrounding environment and is likely described as attention switching (Whittington et al., 1997) which is one of makers showing that individuals focus their attention on the stimulus. A previous study reported (Kisley and Cornwell, 2006) that increase beta oscillation in gamma-to-beta transition process is simultaneously observed with increase gamma oscillation in cases of high enough stimulus intensity.

Beta oscillation is one of neural oscillations in addition to gamma oscillation. It represents activity in range of 12 to 30 Hz. Beta oscillation is normally observed during normal waking state with consciousness and also corresponds to the brain activity in that brain area (Clementz and Blumenfeld, 2001; Karakas and Basar, 1998). For example, beta oscillation over motor cortex are associated with muscle contraction and over prefrontal cortex reflects focus or selective attention, thinking process, and problem solving. Beta oscillation is also a marker of gamma-to-beta transition process to illustrate selective attention to the stimulus in sensory gating. It is known that beta oscillation can be split into 3 groups according to frequency: beta1 (12–15 Hz), beta2 (15–18 Hz), and beta3 (18–25 Hz.) oscillations. However, the 25–30 Hz frequency range is also classified as beta oscillation but known as high beta activity. Different ranges of beta oscillation indicate different intensities of brain activity.

Due to the functions of the brain mentioned above, the brain expresses its characteristics oscillation activities depending on its functions or behavioral states. Each behavioral state contributes to specific brain characteristic which induces the brain to response. However, counter process of the responses is also interesting and can be investigated by inducing the brain to display some specific characteristic and, consequently, letting the brain to perform its function. Investigations on the counter process are lacking; therefore, studying of the brain function after inducing, counter process, is needed. This study aims to investigate the brain responses to stimulus and its function during cognitive function, especially memory, utilizing a stimulus to induce some brain characteristics which are gamma and beta oscillations. These 2 oscillations relate to sensory binding, brain function in response to stimuli, attention, and memory. Hypothesizes of this study are as follows: 1) the brain can be induced by the stimulus and displays some specific characters which are gamma and beta oscillations; and 2) cognitive function, especially working memory, is changed after the brain is induced by the stimulus.

The stimulus used in this study is auditory stimulus which is binaural beat. Binaural beat is a beat phenomenon occurring in the brain upon receiving of 2 pure tones that are almost the same in terms of amplitude and intensity but with slightly different frequencies, separately each ear at the same time. The beat is generated at the superior

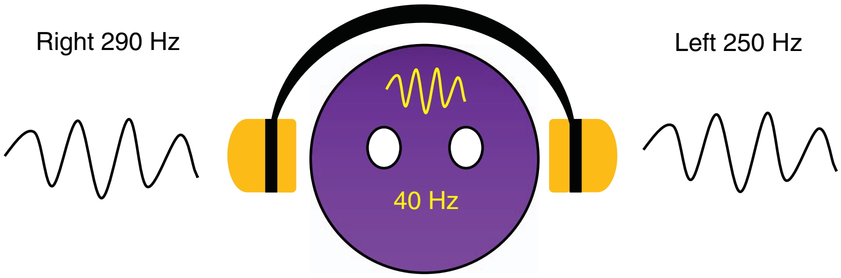

Fig. 1. 40-Hz binaural beat on 250-Hz carrier tone generated in the brain.

olivary complex (Kuwada et al., 1979; Schwarz and Taylor, 2005) which is the first nucleus in central auditory pathway receiving auditory information from both ears. The occurring beat has frequency equals to the differences of the 2 pure tones; for example, 250 Hz pure tone is received by the left ear while 290 Hz pure tone is received by the right ear, 40-Hz binaural beat is perceived in the brain (Fig. 1.) A classical study of binaural beat perception showed that binaural beat with beat frequency of below 35-Hz can be perceived by human perception, but higher beat frequency than 35-Hz are regarded as 2 different sounds (Oster, 1973). However, recent studies have discussed this issue and demonstrated that beat frequency higher than 35-Hz can be perceived by humans; the studies have also noticed 40-Hz beat frequency can also be perceived (Grose et al., 2012; Ross et al., 2014). Therefore, 40-Hz binaural beat, the stimulus in this study, can be perceived by human participants.

One effect of binaural beat is to synchronize firing rate of oscillating brain activity as the binaural beat is perceived, and this effect is called frequency following response (Grose and Mamo, 2012; Worden and Marsh, 1968). After listening to binaural beat, the brain activity related to the beat frequency of the binaural beat is enhanced.

To investigate the proposed hypothesizes, two experiments are setup. The first experiment investigates how the brain responds to 40-Hz binaural beat. This experiment reveals the brain positions at which gamma and beta oscillations are enhanced by the binaural beat. The second experiment demonstrates how behavioral state is manipulated following the brain activity changes after listening to the binaural beat, specifically memory function of cognitive function. This experiment illustrates association between cognitive function and the brain activities induced by the binaural beat.

2. Materials and methods

This study aims to investigate the brain responses to 40-Hz binaural beat, and to investigate the working memory function after induced by 40-Hz binaural beat. The study is composed of 2 experiments, the first experiment evaluated responses of the brain in range of gamma and beta oscillations after listening to 40-Hz binaural beat and the second experiment was working memory task using word list recall task following the listening to 40-Hz binaural beat.

2.1. Participants

Forty-seven participants, 31 males and 16 females, were included for participation in the study. Average age of the participants was 20.6 years old with standard deviation of 1.4 years. All of the participants were normal healthy participants with normal hearing and without neurological disorders. The participants were categorized into 2 groups for the 2 experiments. The first group included 7 participants, and the second group included 40 participants. The objectives and purposes of experiments in the study were described to all participants depending on their experimental group before participation. Alcohol, caffeine, smoking, and medication were not allowed 12 h before the experiments. All participants were asked to complete a consent form prior to participation. All participants were free to withdraw their participation during the experiment for any reasons. The procedures in this study involving human subjects were approved by the Institutional Review Board, Mahidol University with certificate of approval (COA) number 2015/074.1706.

2.2. Experimental room

Room used in this study was sound attenuated with temperature controlled to 25 degree Celsius. The room wall were white for neutral perceptions of emotion and mood as reported (Sroykham et al., 2014). Experimental station was setup in the room as follows: an armchair was placed 3 m from the wall facing to the wall, and height of the armchair was adjustable to each participant. A footrest was placed in front of the armchair at comfort position for each participant.

2.3. Binaural beat stimulus

Stimulus used in this study was 40-Hz binaural beat which was specifically generated for the experiments by delivering 2 nearly identical pure sinusoidal tones with slightly different frequencies. The first tone was 250 Hz presented to the left ear while the other tone was 290 Hz presented to the right ear (Fig. 1.) Using Sound Forge Pro, version 11.0, the 40-Hz binaural beat was created and the stimulus volume was set to 65 dB SPL. The stimulus was delivered to each participant via Monster® Inspiration over-ear headphones.

2.4. Experiment 1: the brain responses to 40-Hz binaural beat in range of gamma and beta oscillations

This experiment was conducted to investigate gamma and beta oscillations responding to 40-Hz binaural beat. Physiological brain responses were the main investigation of the experiment. Electroencephalogram (EEG) was recorded during listening to the stimulus.

2.4.1. Participants

Seven participants, 5 males and 2 females, were included in this experiment. The average age was 22.4 years old with standard deviation of 2.8 years. All participants underwent EEG recording and listening to the stimulus in the experimental room.

2.4.2. EEG setting

Each participant was asked to sit in the armchair in an upright position and relax themselves. The participant's head was measured for fitting electrode cap. The electrode cap, composed of mesh electrodes at the positions corresponding to the international 10/20 system attached to elastic cap, was worn on the participant's head. Conductive get was applied to all electrodes to reduce impedance between scalp and electrode for increasing the quality of signal and decreasing noise. The impedance should be < 5 kΩ. Two electrode cups with conductive paste were clipped on both ear lobules as reference and ground, left and right ear lobules, respectively. Subsequently, all electrodes were paired to a BrainMaster® system for recording EEG signals using BrainMaster Discovery software. Headphones were placed over the participant's ear

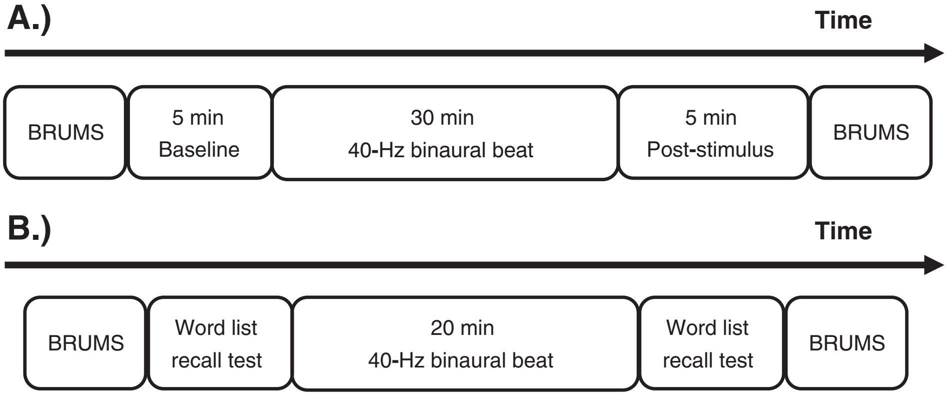

Fig. 2. Experimental procedures; A.) Experiment 1, B.) Experiment 2.

following EEG setup.

2.4.3. Experimental procedures

Prior to including the participants, informed consent form was provided by each participant. Subsequently, participant underwent fulfillment of Brunel Mood Scale (BRMUS) to evaluate emotional state, and then EEG apparatus and headphones were setup.

The BRMUS is a self-report emotional state questionnaire composed of 24 items. These correspond to 6-factor model including ‘Anger’, ‘Confusion’, ‘Depression’, ‘Fatigue’, ‘Tension’, and ‘Vigor.’ Each item can be responded according to 5-point Likert scale which is ranging from 0 to 4 representing ‘not at all’ to ‘extremely’ depending on participant's feeling.

EEG apparatus and headphones were then setup on the participant after completing the BRUMS questionnaire. EEG recording proceeded for consecutive 40 min which comprised 5 min of baseline recording followed by 30 min of 40-Hz binaural beat, and last 5 min of post-stimulus recording. During experimental periods, all participants were asked to focus on the auditory stimulus to keep their arousal state. After post-stimulus recording, the BRUMS was completed once more by the participant to evaluate changes in emotional states (Fig. 2A.)

2.4.4. Data analysis

The recorded EEG signals included 40 min each participant. The power line noise was removed via the 50 Hz bandstop filter prior to the analysis. Every recorded EEG signal was separated as follows: the first 5 min of baseline recording, followed by 6 intervals of 5 min each along experimental period, and the last 5 min of post-stimulus recording. A total of 8 intervals of 5 min each were recorded. Filtered EEG signal of each 5 min interval was carefully selected for 3 min based on the following criteria: clear signal without eyes blinking and motion artifacts, $> 90\%$ reliabilities – both split half and test retest – by NeuroGuide software. Fast Fourier transform (FFT) was conducted on each selected signal to convert the signal from time domain to frequency domain. Absolute power of gamma and beta oscillations were assessed for statistical analysis. Beta oscillation including high beta oscillation was included in the analysis.

2.4.5. Statistical analysis

Paired *t*-tests were conducted to compare the mean of absolute powers of gamma and beta oscillations between each interval and baseline. The comparisons were performed to indicate increasing brain position of FFT absolute power of both gamma and beta oscillations upon listening to the 40-Hz binaural beat. P-values < 0.05 were considered significant. In addition, paired *t*-tests were also conducted to compare the changes in the BRUMS scores before and after listening to the stimulus. However, p-values of < 0.1 were considered different because internal consistency of the BRUMS was reported at 0.90 (McNair et al., 1992).

2.5. Experiment 2: working memory improvement after listening to 40-Hz binaural beat indicated by word list recall task

This experiment was conducted to investigate working memory function followed by the brain stimulation using 40-Hz binaural beat. Word list recall task was utilized to evaluate working memory function.

2.5.1. Participants

Forty participants were involved in this experiment, including 26 males and 14 females. The average age of the participants was 20.3 years old with standard deviation of 0.7 years. All participants underwent the experiment procedures in the experimental room.

2.5.2. Word list recall task

Word list recall task is a memory task that presents words to individuals, each word is presented for specific periods of time. After the last word finishes, the individuals are required to recall all the words that they could remember. Each position of the word represents different brain functions used to remember that word. The beginning of the list was strongly associated with primacy effect which is an effect associated with increased attention level while the end of the list was related to buffer at the recall period (Gavett and Horwitz, 2012).

The word list recall task in this study was composed of 2 sets of words (Supplementary Table 4.) Each word list included 15 word items established from 3 to 7 alphabets, and all words were presented for 2 s via monitor positioned in front of the armchair. The words appeared and disappeared with time without labeled numbers. The recall period began two minutes from the time the last word finished. The participants were asked to write down every word they could remember in any order.

2.5.3. Experimental procedures

The same procedures as the first experiment for invitation participants were performed to all participants in this experiment. Informed consent form was completed by all participants after objectives and purposed were described.

The same experimental room was used but a desk with a monitor was included in this experiment. The desk and monitor were placed in front of the armchair for word list recall test. Prior to listening to the 40-Hz binaural beat, the BRUMS questionnaire was completed by participant. Then, the first word list recall task was conducted to the participant. Subsequently, the binaural stimulus was provided to participant for 20 min without EEG signal recording which time the monitor was dark. When the stimulus had finished, the second word list recall task using another set of words was performed to the participant. The experiment finished upon completing the BRUMS questionnaire again to evaluate changes in emotional states after listening to the stimulus (Fig. 2B.)

2.5.4. Statistical analysis

McNemar's test was conducted to compare differences in words recalled by the participant before and after listening to the stimulus. The recalled words were analyzed in a position by position fashion regarding which word position corresponded to increased recall percentages. The comparison was calculated to indicate increased working memory after listening to 40-Hz binaural beat. P-value < 0.05 was considered significantly different. In addition, paired *t*-tests were conducted on the average number of words recalled by participants and the average points of total words that the participants could recall before and after listening to the stimulus. Each word position had its own weighting point as follows: positions 1 to 3 at the beginning and end of the list were allocated 1 point each; positions 4 to 6 at the beginning and end of list were allocated 3 points each; and positions 7 to 9 in the list were allocated 5 points each. Moreover, similar to the first experiment, paired *t*-tests were also performed on the BRUMS scores to evaluate changes in emotion states before and after listening to the stimulus. Significant difference was considered for p-values < 0.1.

3. Results

Gamma and beta oscillations were assessed after listening to the 40-Hz binaural beat for 30 min. Cognitive function, specifically working memory, was also investigated to determine whether it changed after brain activities were induced to express some specific activity upon exposure to the 40-Hz binaural beat for 20 min. Results were shown separately for each experiment.

3.1. Experiment 1: the brain responses to 40-Hz binaural beat in range of gamma and beta oscillations

3.1.1. Baseline recording

During baseline recording, absolute power of gamma oscillation was expressed the highest value at frontal region of the brain, especially on the right hemisphere, while occipital and central regions displayed lower values (Fig. 3.) Beta oscillation exhibited opposite pattern in that, the maximum value occurred at the occipital region of the brain which was dominant on the right side while the rest of regions showed widespread lower values throughout (Fig. 4.) However, the trend in absolute power of high beta oscillation was similar to gamma oscillation, showing the highest value at right frontal region of the brain and low values throughout the rest of brain regions (Fig. 5.)

3.1.2. Gamma oscillation

After the participants had listened to the 40-Hz binaural beat, the FFT absolute power of gamma oscillation increased with time, especially at frontal and central regions. The increased FFT absolute power of gamma oscillation was enhanced by the stimulus for a period of time (Fig. 3.) After that period, the value was gradually decreased (Supplementary Fig. 1.) This observation was also clearly found at frontal region of the brain. Significant differences in FFT absolute value of gamma oscillations with time were expressed (Table 1.) The greatest induced changes were found within 15 min of listening (Table 2), especially at frontal and central regions; therefore, the duration of the greatest changes was 15 min. However, 20 min also presented some significant differences. Beyond this 20 min, the no marked changes in gamma oscillation occurred. Average FFT of absolute powers of gamma oscillation at F4 and Fp2 were shown in Fig. 6A and B, respectively. F4 and Fp2 were shown because all neural oscillations exhibited changes at these 2 positions. The rests of average FFT absolute power of gamma oscillation were shown in Supplementary Fig. 1; standard deviations of all paired differences are shown in Supplementary Table 1.

3.1.3. Beta oscillation

The FFT absolute power of beta oscillation in baseline recording was not similar to that of gamma oscillation; however, the changes in FFT absolute power of beta oscillation expressed similar trend to gamma oscillation. Frontal region exhibited increased FFT absolute power of beta oscillation while the rest of regions gradually decreased over time, especially at occipital region that the value dramatically decreased (Fig. 4.) Significant differences in the FFT absolute power of beta oscillation with time were illustrated (Table 3.) The greatest changes in induced positions were in frontal region within 15 min of listening (Table 4.) Moreover, similar to gamma oscillation, beta oscillation appeared to be not significantly induced after 20 min. Average FFT of absolute powers of beta oscillation at F4 and Fp2 were shown in Fig. 6C and D, respectively. The rests of average FFT absolute power of beta oscillation were shown in Supplementary Fig. 2; standard deviations of all paired differences are shown in Supplementary Table 2

3.1.4. High beta oscillation

Similar trend to gamma oscillation appeared for high beta oscillation corresponding to the maximum FFT absolute power at the right frontal regions of the brain. After listening to the 40-Hz binaural beat, high beta oscillation increased in widespread regions of the brain over

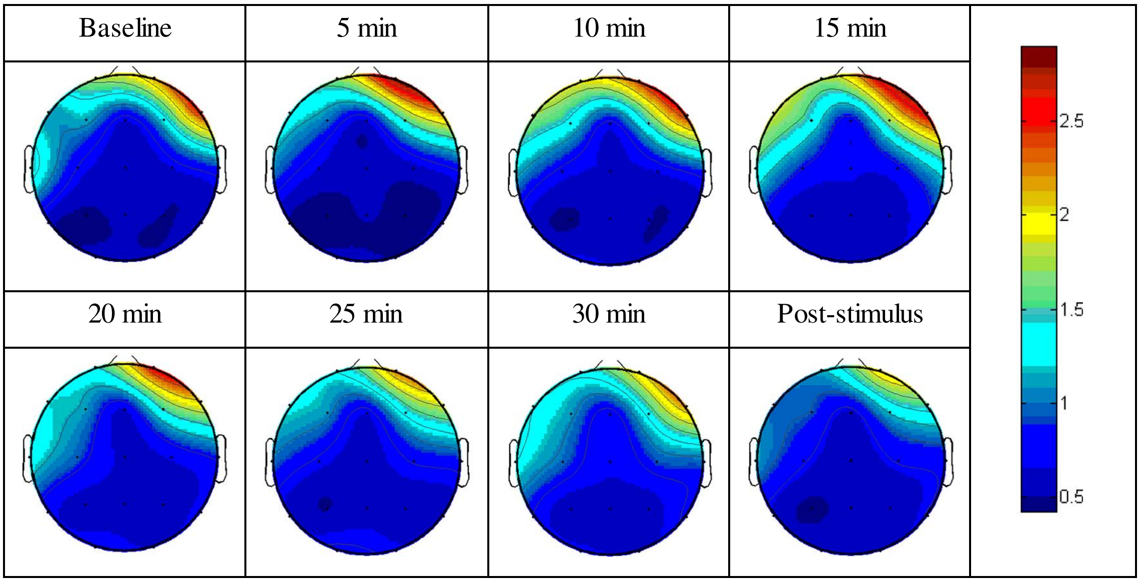

Fig. 3. Brain topographic mapping of gamma oscillation from baseline to post-stimulus.

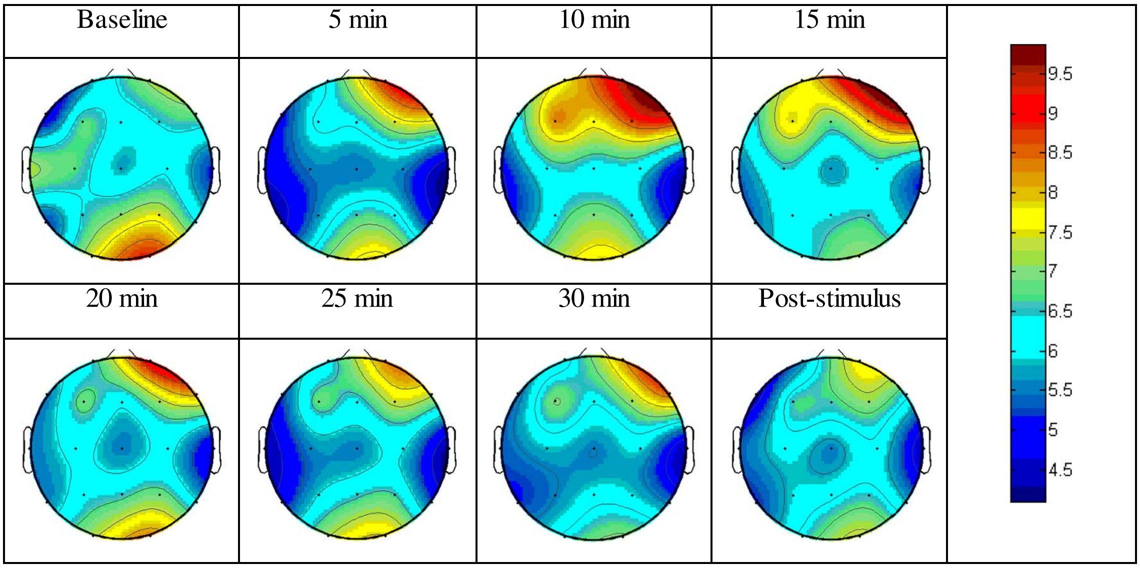

Fig. 4. Brain topographic mapping of beta oscillation from baseline to post-stimulus.

time, especially in the right frontal region. Central region was also enhanced in high beta oscillation as time passed while the rest of regions were seemingly not increased. Listening duration also affected the induced high beta oscillation, and the FFT absolute power of high beta oscillation was shown (Fig. 5.) Significant differences in the FFT absolute power of high beta oscillation were displayed (Table 5.) The greatest changes in positions of brain regions induced by the stimulus were predominant in central region within 15 min of listening (Table 6.) Within 20 min of listening, high significance remained present; however, over 20 min of listening, the differences became less significant. Average FFT of absolute powers of high beta oscillation at F4 and Fp2 were shown in Fig. 6E and F, respectively. The rests of average FFT absolute value of high beta oscillation were shown in Supplementary Fig. 3; standard deviations of all paired differences are shown in Supplementary Table 3.

Table 1
Significantly different brain positions of the absolute power of gamma oscillation in each interval duration compared to baseline.

Listening interval	Electrode position
5 min	Fp2, F4
10 min	Cz
15 min	Fz, F4, T6, Cz
20 min	F4
25 min	N/A
30 min	N/A
Post stimulus	N/A

3.1.5. Brunel mood scale after listening to 40-Hz binaural beat for 30 min

After listening to the stimulus, the Brunel Mood Scale was completed by each participant to evaluate emotional states following listening. Comparisons between before and after listening indicated changes in emotional states. Significant changes both increases and decreases were presented (Table 7.) Fatigue factor was the only factor that significantly increased. Worn out and sleepy were fatigue factor that increased after listening to the stimulus comparing to before listening. Other emotional states did not show any significant changes, neither increases nor decreases.

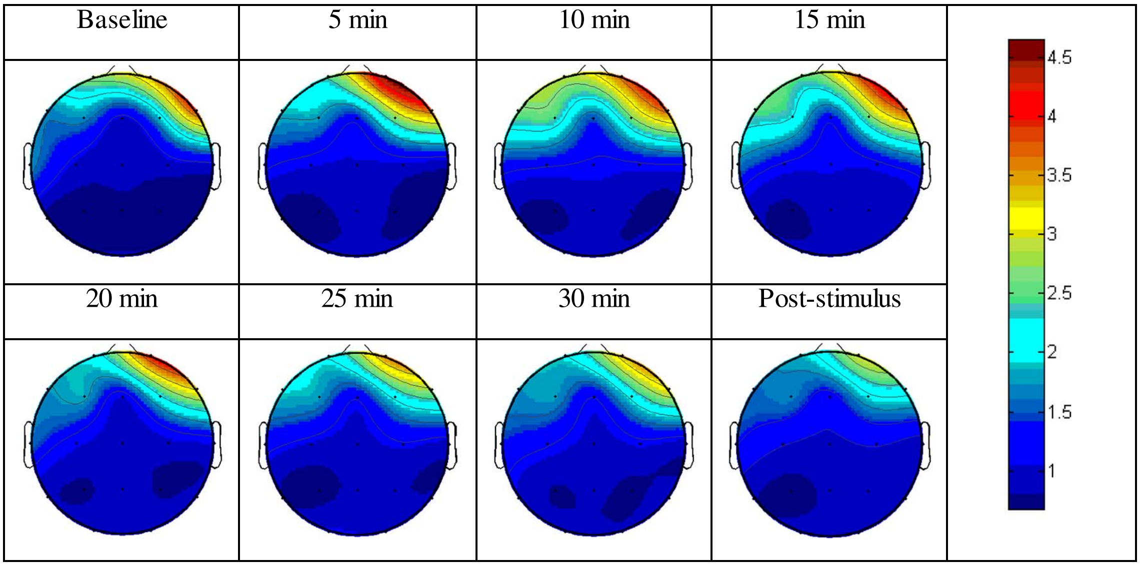

Fig. 5. Brain topographic mapping of high beta oscillation from baseline to post-stimulus.

Table 2
Paired *t*-test analysis of the absolute power of gamma oscillation of 15 min duration compared to baseline. (Data are shown only significant differences.)

Channel	15 min	Baseline	Paired differences			P-value
			Mean	Std. deviation	Std. error mean	
Fz	0.7574	0.6601	0.0973	0.1120	0.0423	0.0306
F4	1.6929	1.2691	0.4237	0.5271	0.1990	0.0388
T6	0.8188	0.5778	0.2410	0.3269	0.1235	0.0495
Cz	0.6816	0.5705	0.1111	0.1083	0.0409	0.0174

Table 3
Significantly different brain positions of the absolute power of beta oscillation in each interval duration compared to baseline.

Listening interval	Electrode position
5 min	Fp2
10 min	Fp1, Fp2, F8
15 min	Fp1, F7, Fp2, F4, F8
20 min	Fp2, F4, F8
25 min	Fp2
30 min	Fp2
Post stimulus	N/A

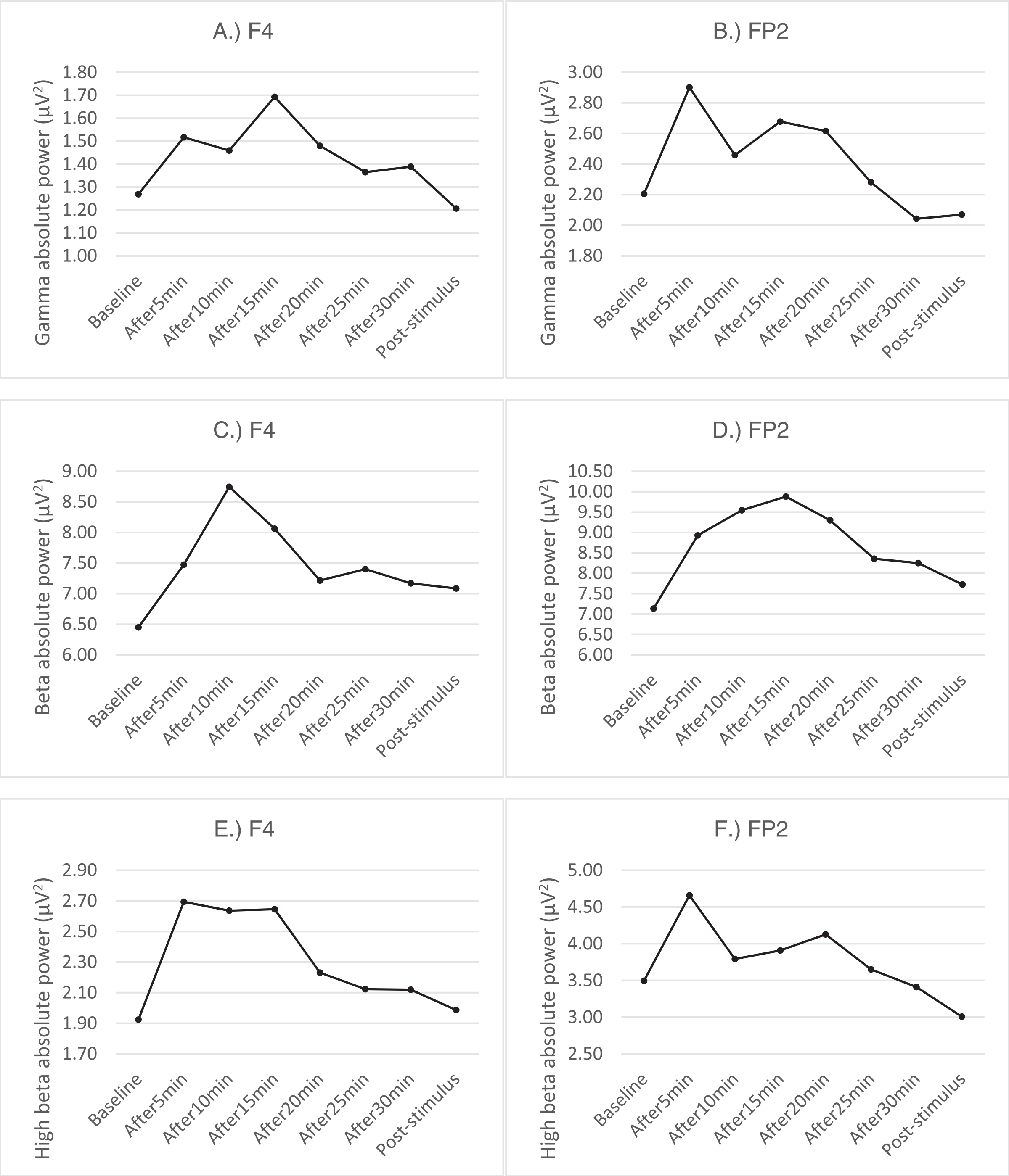

Fig. 6. Average FFT of absolute power of neural oscillations at F4 and FP2 electrode positions from baseline to post-stimulus after listening to 40-Hz binaural beat; A.) and B.) were gamma oscillation, C.) and D.) were beta oscillation, E.) and F.) were high beta oscillation.

Table 4
Paired *t*-test analysis of the absolute power of beta oscillation of 15 min duration compared to baseline. (Data are shown only significant differences.)

Channel	15 min	Baseline	Paired differences			P-value
			Mean	Std. deviation	Std. error mean	
Fp1	7.6104	5.7838	1.8266	2.0378	0.7702	0.0277
F7	6.4867	4.5072	1.9794	2.6484	1.0010	0.0477
Fp2	9.8784	7.1345	2.7439	1.2100	0.4574	0.0005
F4	8.0625	6.4502	1.6123	1.7229	0.6512	0.0240
F8	9.6422	6.8474	2.7948	2.5990	0.9823	0.0147

Table 5
Significantly different brain positions of the absolute power of high beta oscillation in each interval duration compared to baseline.

Listening interval	Electrode position
5 min	Fp2, F4
10 min	F3, C3, P3, Fz, Cz, Pz
15 min	C3, P3, F4, C4, Cz, Pz
20 min	P3, F4, Pz
25 min	N/A
30 min	P3, Cz
Post stimulus	N/A

Table 6
Paired *t*-test analysis of the absolute power of high beta oscillation of 15 min duration compared to baseline. (Data are shown only significant differences.)

Channel	15 min	Baseline	Paired Differences			P-value
			Mean	Std. deviation	Std. error mean	
C3	1.2063	1.0108	0.1956	0.1824	0.0689	0.0148
P3	0.7581	0.6766	0.0815	0.0770	0.0291	0.0156
F4	2.6454	1.9240	0.7214	0.9743	0.3683	0.0489
C4	1.1741	0.9625	0.2116	0.2818	0.1065	0.0471
Cz	1.1044	0.8570	0.2474	0.2423	0.0916	0.0178
Pz	0.9209	0.7751	0.1458	0.1911	0.0722	0.0450

Table 7
Average ranges of the BRUMS scores for 24 items before and after listening to 30 min of the 40-Hz binaural beat in experiment 1 and the significant increases (arrow up) and decreases (arrow down.) S.D. indicated standard deviation of paired differences ($\overline{d}$).

Factors	Items	Before	After	S.D.	Significance
Tension	Panicky	0.0000	0.0000	0.0000	
	Anxious	0.1429	0.0000	0.3780	
	Worried	0.1429	0.2857	0.3780	
	Nervous	0.1429	0.1429	0.0000	
Anger	Annoyed	0.0000	0.0000	0.0000	
	Bitter	0.0000	0.0000	0.0000	
	Angry	0.0000	0.1429	0.3780	
	Bad tempered	0.0000	0.1429	0.3780	
Depression	Depressed	0.0000	0.0000	0.0000	
	Downhearted	0.0000	0.0000	0.0000	
	Unhappy	0.0000	0.0000	0.0000	
	Miserable	0.0000	0.0000	0.0000	
Fatigue	Worn out	0.4286	0.7143	0.4880	↑
	Exhausted	0.4286	0.5714	0.6901	
	Sleepy	0.8571	1.7143	1.5736	↑
	Tired	0.4286	0.5714	0.6901	
Vigor	Lively	0.4286	0.5714	0.8997	
	Energetic	0.2857	0.5714	0.7559	
	Active	0.2857	0.4286	0.8997	
	Alert	0.4286	0.8571	1.1339	
Confusion	Confused	0.0000	0.1429	0.3780	
	Muddled	0.0000	0.1429	0.3780	
	Mixed up	0.0000	0.0000	0.0000	
	Uncertain	0.1429	0.1429	0.0000	

3.2. Experiment 2: working memory improvement after listening to 40-Hz binaural beat indicated by word list recall task

3.2.1. Word list recall task

Fig. 7A demonstrated recall percentages of each word in the list position-by-position before and after listening to the stimulus for 20 min. The average number of the words recalled by the participants before and after listening to the stimulus were also shown (Fig. 7B.) Based on the respective points of each position, average points of the words recalled by the participants was calculated (Fig. 7C.) Before listening to the stimulus, trends in the recall percentages expressed the highest value at the beginning of the list and gradually decreased by position. The minimum recall percentages appeared in the middle of the list and gradually increased toward the end of the list. Similar trend also occurred after listening period; but at the word number 8 which was the middle of the list, the recall percentage dramatically increased compared to before listening to the stimulus (Fig. 7A.) McNemar's test revealed significant difference only at the word number 8. Another one position trends to demonstrate higher recall but no significance reaches was the word number 11. The average numbers of words recalled by each participant before and after listening to the stimulus were not significantly different. Upon weighting the words based on position, the average recall points were not significantly different before and after listening to the stimulus. However, the recall of word number 8 was dramatically significant with p-value < 0.01.

3.2.2. Brunel mood scale after listening to the 40-Hz beat frequency of binaural beat for 20 min

Table 8 demonstrated the significant increases or decreases in emotional states after listening to the stimulus compared to before listening to the stimulus. Significance was considered by p-value < 0.1; however, double arrows in the table indicated p-value < 0.05. For the changes in emotional states evaluated by the BRUMS, the same factors presented the same trend, either increasing or decreasing. Those factors were tension, confusion, depression, and vigor. Tension, confusion, and depression significantly decreased while vigor significantly increased. Tension exhibited the maximum changes as 3 of the 4 items were significantly decreased after listening compared to before listening to the stimulus. However, there were 2 factors demonstrating both increasing and decreasing significances in the same factors including anger and fatigue. After listening, annoyed item of anger significantly increased while bad tempered decreased. Likewise, sleepy item of fatigue significantly decreased while worn out increased. The changes in emotional states were in accordance with one another.

4. Discussion

This study seeks to investigate the brain activity changes in gamma and beta oscillations after listening to 40-Hz binaural beat and to investigate cognitive function, i.e., working memory function followed by the induced brain activity after listening to the same stimulus. Two experiments were devised to examine these aims. In the first experiment, EEG signals were recorded before, during, and after listening the 40-Hz binaural beat for 30 min. Fast Fourier transform was conducted to recorded EEG signals. In the second experiment, word list recall task which is a task used to evaluate working memory was completed by the participants before and after listening to the 40-Hz binaural beat. However, the listening durations differed between the 2 experiments, corresponding to 30 and 20 min of listening durations, respectively. Brunel Mood Scale (BRUMS) was conducted to evaluate emotional states of the participants before and after listening to the stimulus in both experiments.

The findings of experiment 1 indicate that listening to the 40-Hz binaural beat enhances gamma oscillation in temporal, frontal, and central region. After 15 min of listening, gamma oscillation is markedly induced at several regions, as mentioned. This enhancement seemingly

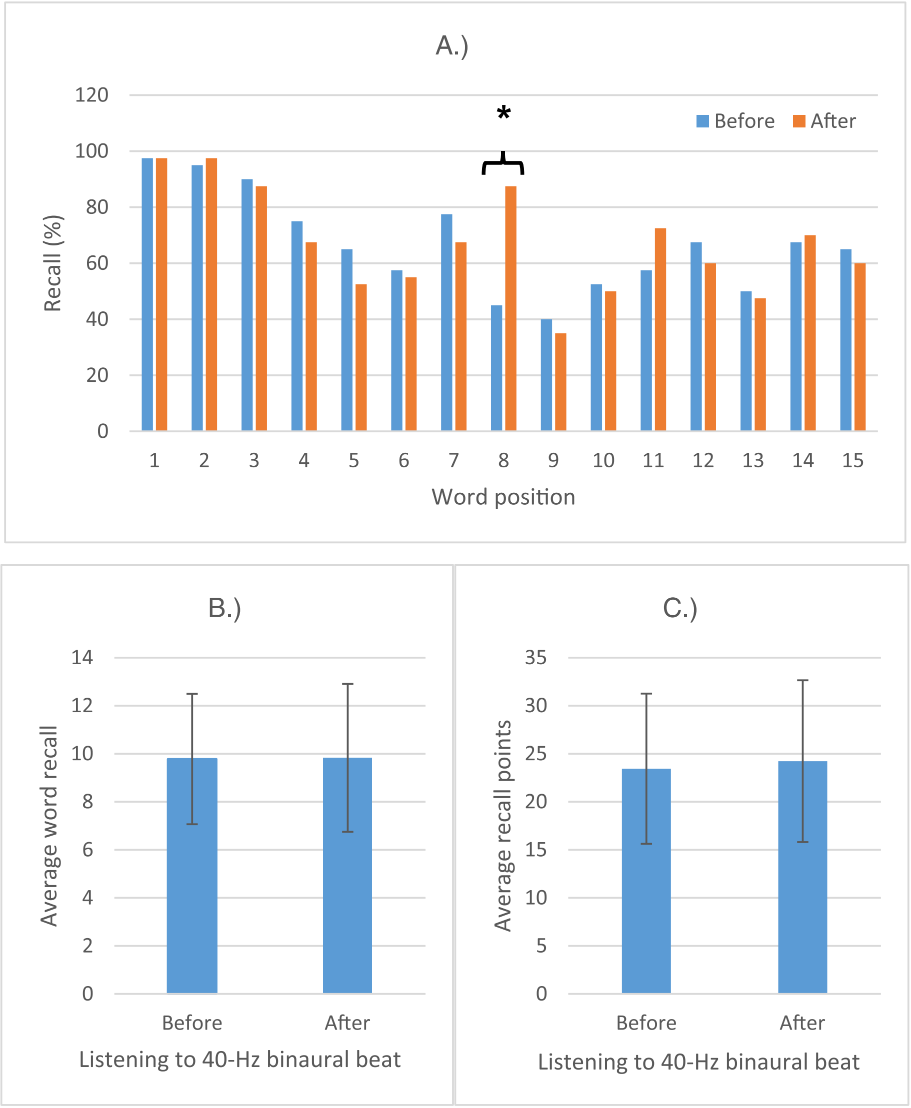

Fig. 7. Word list recall results; A.) Recall percentage of each word position in word list recall test (* indicates significant difference), B.) Average words recalled by participants before and after listening to 40-Hz binaural beat, C.) Average point calculated from the weight factor of each word position in word list recall test before and after listening to 40-Hz binaural beat.

illustrates that the right hemisphere responds to binaural beat more than left hemisphere. The enhanced gamma oscillation observed in several areas could support frequency following response effect (Grose and Mamo, 2012; Worden and Marsh, 1968). This interesting effect can be explained as follows: after listening to binaural beat, the neural oscillation trends to synchronize in frequency range related to the received beat frequency. In this study, 40-Hz binaural beat is in range of gamma oscillation which exhibited enhanced trend.

Gamma oscillation is a brain activity which maintains brain arousal and carries schema to communicate between brain areas (Fries, 2005; Llinas et al., 2002). The increased gamma oscillation in the findings reveals brain areas that responds to auditory stimulus which are composed of temporal, frontal, and central regions. These responses are associated with the cooperation of several brain areas for processing the perception of auditory stimulus after stimulus is received. This mentioned process is so-called binding process or sensory integration (Harris et al., 2009; Vermeire et al., 2016; Martin and Jerger, 2005), a process by which the brain evaluates received stimulus at higher level of perception. In other words, stimulus information received by receptor is carried to primary sensory areas related to that stimulus. After that to perceive detailed features of the stimulus, associated areas are required to analyze the stimulus. The combined processing of several brain areas to analyze the stimulus is known as binding process or sensory integration. Therefore, the increased gamma oscillation after listening to the 40-Hz binaural beat not only supports the frequency following response of binaural beat but also seems to reveal the brain areas related to auditory processing of sensory integration. This finding is in agreement with a previous study on sensory integration which investigated the brain responses to visual stimulus (Muller et al., 2000). The previous study showed that several brain areas responded to visual stimulus by exhibiting of gamma oscillation in associated brain areas following visual stimulus.

Other oscillations in addition to gamma oscillation include beta and high beta oscillations which were investigated as oscillations of interest in this study. The findings of beta oscillation indicated that increased beta oscillation trends to exhibit in frontal region of both hemisphere. As discussed, the binding process of auditory stimulus leads to enhancement of gamma oscillation in several associated areas. Furthermore, those gamma oscillations are substituted by beta oscillation if the intensity of stimulus is high enough. This phenomenon is so-called gamma-to-beta transition (Kisley and Cornwell, 2006). However, this finding is agreeable with several studies stating that beta oscillation was exhibited in associated areas while gamma oscillation was increased in specific area to the stimulus (Barth and MacDonald, 1996; Pantev, 1995; Pantev et al., 1991; Roelfsema et al., 1997; Von Stein et al., 1999), and that gamma and beta oscillations can be observed within the same period (Kisley and Cornwell, 2006). Interestingly, in these findings, beta oscillation increased primarily in frontal region

Table 8
Average ranges of the BRUMS scores for 24 items before and after listening to 20 min of the 40-Hz binaural beat in experiment 2 and the significant increases (arrow up) and decreases (arrow down.) S.D. indicated standard deviation of paired differences ($\overline{d}$).

Factors	Items	Before	After	S.D.	Significance
Tension	Panicky	0.7250	0.7250	1.1983	
	Anxious	0.4500	0.3250	0.5633	↓
	Worried	0.5500	0.2750	0.7841	↓↓
	Nervous	0.6250	0.3500	0.8469	↓↓
Anger	Annoyed	0.3750	0.6750	1.0908	↑↑
	Bitter	0.1750	0.1750	0.3203	
	Angry	0.0750	0.0500	0.2762	
	Bad tempered	0.3750	0.2250	0.6222	↓
Depression	Depressed	0.3750	0.2250	0.6222	↓
	Downhearted	0.3000	0.2250	0.5723	
	Unhappy	0.2000	0.1750	0.2762	
	Miserable	0.2250	0.1000	0.4043	↓↓
Fatigue	Worn out	1.1250	1.3500	1.0497	↑
	Exhausted	1.6750	1.6750	1.0127	
	Sleepy	2.0000	1.5000	1.3397	↓↓
	Tired	1.5500	1.5500	1.0127	
Vigor	Lively	1.2250	1.4250	1.0178	
	Energetic	0.9000	1.2250	1.1410	↑↑
	Active	1.0000	1.1000	0.9819	
	Alert	0.9000	1.1750	1.2606	↑
Confusion	Confused	0.8250	0.6750	0.8638	
	Muddled	1.1750	0.8000	0.9524	↓↓
	Mixed up	1.0500	0.8500	1.0178	
	Uncertain	1.0500	0.8500	0.7910	↓

while gamma oscillation was enhanced in temporal, frontal, and central regions. Moreover, high beta oscillation, which is a beta oscillation with higher frequency that exhibits more intense functions in the brain areas where high beta oscillation occurs, illustrates increased absolute power in frontal and central regions of the brain. This finding supports the binding process and gamma-to-beta transition and indicates that those brain areas are active for binding features of the stimulus. Central region including parietal region is connection area among several brain areas; therefore, the increases in gamma, beta, and high beta oscillations after listening to the 40-Hz binaural beat cloud be explained by the binding process or sensory integration and gamma-to-beta transition.

Beta oscillation is an oscillation indicating brain activity of that brain area so frontal and central regions show potentially high activities because both regions exhibit beta and high beta oscillations. Frontal region of the brain functions primarily in thinking, problem solving, regulating activities, paying attention, emotional control, and other functions related to consciousness including learning. Therefore, the increases in beta and high beta oscillations at the frontal region point out that its functions can be improved due to the higher activity.

The results of neural oscillations in the experiment 1 demonstrated that most brain areas were induced within 15 min, and therefore, 20 min was extreme duration ensuring the induction of neural oscillation. Therefore, 20 min of listening is utilized in the experiment 2.

The findings of experiment 2 suggest that working memory can be improved by listening to the 40-Hz binaural beat. Working memory function is evaluated by word list recall comparing performance between before and after listening to the 40-Hz binaural beat. Literature has suggested that there are two related cognitive abilities that affect word list recall test: attention, and working memory (Gavett and Horwitz, 2012; Hogervorst et al., 2004; MacLeod and Kampe, 1996). However, there is another ability which is latent buffer of the brain for memorizing recent word. One study stated that the word position in word list recall test is affected by different cognitive abilities (Gavett and Horwitz, 2012). Initial positions of the list indicate attention level while ultimate positions indicate latent buffer of the brain. Therefore, the middle positions of the list correspond to working memory function. The current findings reveal that working memory increases after listening to the 40-Hz binaural beat regarding to increase in recalled word at number 8. Initial and ultimate positions do not show any difference. However, a previous study stated that not all word positions in the list are appropriate in memory ability evaluation. The current finding is generally consistent with that study including trend regarding to the finding of each word position, where the recall of the word at the position corresponding to working memory increase dramatically.

The Brunel Mood Scale (BRUMS) disseminates emotional states of the participants in both experiments, differently. In experiment 1, fatigue factor increase in both worn out, and sleepy items. This points out that listening to long duration of the 40-Hz binaural beat leads to over activation and causes the brain to fatigue. Unsurprisingly, if something is stimulated for long duration, fatigue may occur. Therefore, listening to this binaural beat stimulus for over 20 min may cause over stimulation of the brain. On the other hand, experiment 2 shows that there are both increases and decreases in emotional states depending on each factor. Tension, anger, depression, fatigue, and confusion factors illustrate decreases in emotional states after listening to the stimulus for 20 min, whereas vigor factor, which represents to be powerful, increases. However, these two findings are generally compatible with each other, although they seem to differ because listening durations are different. Duration that is too long can lead to negative effects; for example, increase of fatigue. Likewise, a suitable duration can lead to positive effects; for example, decreases of tension and depression while increase of powerful energy. These results support that emotional states are changed upon listening of the stimulus, and the duration of stimulus affects to these changes.

Moreover, the changes in emotional states due to the stimulus seemingly relate to the induced neural oscillations. Observations of the neural oscillations in experiment 1 and 2 revealed that different neural oscillations lead to different emotional states. The findings of experiment 2 indicate that beta, and high beta oscillations are dramatically higher after listening to the stimulus in several brain regions. Beta and high beta oscillations are normally found during consciousness, especially when the brain is active; therefore, the significantly higher vigor factor of the BRUMS is consistent with the neural oscillation. Furthermore, negative emotional states are also lower after listening which generally corresponds to induced neural oscillations. However, the findings of experiment 1 reveal increase of fatigue factor resulting from too long duration of stimulation. In addition, during the post-stimulus period of experiment 1, no increases in absolute power of gamma, beta, and high beta oscillations are found, and interestingly, the absolute powers of those oscillations seemingly appear to be lower than baseline period – prior to listening to the stimulus. These observations support changes in emotional states associated with the neural oscillations.

The two hypothesizes of this study are potentially confirmed by the experiments. Neural oscillations can be induced by the 40-Hz binaural beat which are indicated by gamma oscillation in related areas. Moreover, beta and high beta oscillations displayed in those areas support that gamma-to-beta transition of the oscillations while gamma oscillation also presents after listening. Interestingly, working memory function is improved due to the neural oscillation in beta and high beta ranges which is indicated by word list recall task, and changes in emotional states are related to the induced neural oscillations. These results seemingly show the 40-Hz binaural beat can modulate brain functions.

4.1. Limitations

This study provides insight into acute effects on cognitive functions for both working memory and emotional states due to binaural beat stimulation using immediate recall as an indicator. Therefore, a limitation of this study is that we investigated only acute effects of the stimulus on brain functions. In order to evaluate improvements in cognitive functions for long duration, especially working memory, and to investigate development of habituation, experiments incorporating training sessions are required.

5. Conclusion

Listening to 40-Hz binaural beat can induce gamma oscillation in several brain areas. Those induced areas are related to auditory response of the brain. Moreover, beta and high beta oscillations are also induced by gamma oscillation in several associated areas, particularly frontal region. These patterns of neural oscillations improve working memory function as reflected by word list recall task; in addition, emotional states are modified relating to induced neural oscillations.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijpsycho.2017.07.010.

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