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Analysis of EEG activity in response to binaural beats with different frequencies

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ABSTRACT

When two coherent sounds with nearly similar frequencies are presented to each ear respectively with stereo headphones, the brain integrates the two signals and produces a sensation of a third sound called binaural beat (BB). Although earlier studies showed that BB could influence behavior and cognition, common agreement on the mechanism of BB has not been reached yet. In this work, we employed Relative Power (RP), Phase Locking Value (PLV) and Cross-Mutual Information (CMI) to track EEG changes during BB stimulations. EEG signals were acquired from 13 healthy subjects. Five-minute BBs with four different frequencies were tested: delta band (1 Hz), theta band (5 Hz), alpha band (10 Hz) and beta band (20 Hz). We observed RP increase in theta and alpha bands and decrease in beta band during delta and alpha BB stimulations. RP decreased in beta band during theta BB, while RP decreased in theta band during beta BB. However, no clear brainwave entrainment effect was identified. Connectivity changes were detected following the variation of RP during BB stimulations. Our observation supports the hypothesis that BBs could affect functional brain connectivity, suggesting that the mechanism of BB–brain interaction is worth further study.

1. Introduction

When a sound with a steady intensity and frequency was presented to one ear and another with the same intensity but slightly different frequency, the brain would produce pulsations in the amplitude and localization that is the same with the perceived sounds, which are known as "binaural beat" or "binaural tone" (Dove, 1841; Oster, 1973).

The binaural beat (BB) has a fundamental frequency and a modulation frequency. For example, if a pure tone of 550 Hz was displayed in one ear and 560 Hz in the other, the BB would have a fundamental frequency of $(550 + 560)/2 = 555$ Hz with a modulation frequency of 10 Hz. The frequency difference between the two sounds must be small ($\leq$30 Hz) for the BB to occur; otherwise, the two tones would be captured by the two ears separately, and no beat would be perceived. It was suggested that tones with a frequency from 200 to 900 Hz were more effective in provoking BB than those exceed 1000 Hz (Licklider et al., 1950; Wahbeh et al., 2007; Pratt et al., 2010). Moreover, the probability of detecting the BB was maximized around 500 Hz (Perrott and Nelson, 2005).

It has been reported that many physiological and psychological processes can be altered by BB. For example, it can help one to reduce self-reports of anxiety, to deepen relaxation meditation and to improve hypnotic susceptibility (Le Scouarnec et al., 2001; Lavallee et al., 2011; Brady and Stevens, 2000). In addition, BB can help one to relax (Foster, 1990) and improve one's memory ability, as well as alertness and vigilance status. BB is also helpful in mental concentration and psychomotor performance, leading to good feelings (Kennerly, 1996; Lane et al., 1998; Sornson, 1999). With all those importance in application, however, agreements have not been reached regarding the mechanism of BB. Some researchers (such as Brady and Stevens, 2000; Schwarz and Taylor, 2005; Karino et al., 2006) tended to believe the hypothesis of brainwave entrainment effect or also known as Frequency Follow Response (Marsh et al., 1970), which is similar to what will occur during stimuli such as light oscillating at a stable frequency or acoustics such as human speech or consistent tones (Silberstein et al., 1990; Aiken and Picton, 2008; Krishnan et al., 2004).

The basic theoretical assumption was that the human brain had a tendency to change its dominant EEG frequency towards the frequency of external stimulus by entraining the brain to synchronize neural activity with BB stimuli or other external stimulations. However, other studies did not achieve similar observations (Stevens et al., 2003; Wahbeh et al., 2007; Goodin et al., 2012; Vernon et al., 2012). Goodin et al. (2012) assessed theta (7 Hz) and beta (16 Hz) bands in 2-min BB carrier tone. They reported no significant differences in cortical frequency power during the period of BB stimulation compared to using a white noise signal. Consistently, Vernon et al. (2012) found no significant change in EEG from BB stimulation with alpha (10 Hz) and

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beta (20 Hz) frequencies. One of the reasons that the power of EEG did not change in these studies might be their short-duration of stimulation (ten 1-minute segments). Another reason may be that the 'averaging' processor that they used in EEG power calculation over a long period of time wiped out possible changes. Besides the brainwave entrainment effect, there might be other hypothesis for the mechanism of behavior and cognition changes under BB stimulations. For example, functional connectivity of the brain, not just brainwave oscillation, may change under BB stimulation. To evaluate the functional connectivity of different brain regions, we calculated the phase-locking value (PLV), and cross-mutual information (CMI) between different cortical areas, using EEG signals. CMI and PLV have been widely used to study the brain functions in complex diseases such as schizophrenics, Alzheimer's disease (AD) and self-determinant motor task (Na et al., 2002; Jeong et al., 2001; Lu et al., 2011) or cognitive tasks (Lehmann et al., 2006; Rose and Büchel, 2005). Although mechanisms of stimuli induced short-lasting functional connectivity changes of the brain have been studied using EEG or MEG signals, there has been no report studying the information transmission variations in brain networks using BB stimulation.

Different to previous studies, we extended the duration of BB stimulations to 5 min to study possible induced brainwave entrainment effect. Moreover, we focused on the changes of relative power (RP) instead of power, and tracking the EEG changes over time, instead of 'averaging' it, to avoid losing information. In addition, we used new methods—PLV and CMI—to test the connectivity variations during BB stimulations.

2. Materials and methods

2.1. Subjects

We recruited 13 right handed healthy subjects from Tianjin University (6 male and 7 female, aged from 19 to 26). The subjects were informed that they cannot take any products containing caffeine, alcohol or drugs during the week before the experiment. In addition, they were informed that they would hear several sound groups, during which they were required to be quiet and concentrate. However, they were not noted about the sequence of BBs and purpose of the study. All subjects were reported with no history of mental illnesses or attention deficit disorders.

2.2. EEG signal acquisition

Previous studies suggested that BB would be perceived more clearly when the carrier tones were closer to 500 Hz (Perrott and Nelson, 2005). Hence, the BBs and pink noise were created by Adobe Audition with a carrier tone at 550 Hz. The delta BB recording contained two sinusoidal tones, left at 550 Hz and right at 551 Hz, to produce a 1 Hz delta BB. The frequencies were 550 Hz and 555 Hz for generating the 5 Hz theta BB, 550 Hz and 560 Hz for 10 Hz alpha BB and 550 Hz and 570 Hz for the 20 Hz beta BB. All stimuli were played to subjects at 70 dB SPL, which was within the range recommended by Stevens et al. (2003). In order to avoid influence of any external noise, a sound isolating earphone was used.

The EEG acquisition equipment and the signal analysis system were manufactured by NeuroScan Company. The brain regions were parcellated into six areas: frontal (Fp1, Fp2, F3, Fz and F4), left temporal (F7, T3 and T5), right temporal (F8, T4 and T6), central (C3, Cz and C4), parietal (P3, Pz and P4) and occipital (O1 and O2) areas. Those areas were numbered from I to VI as shown in Fig. 1(a). Scalp potential of each channel was amplified separately, band-pass filtered (0.5–70 Hz), and digitized with a sample rate of 1000 Hz.

The experiment was conducted in a shielded room. During the process, the subjects wore the same headphones. Experimental diagram was provided in Fig. 1(b). In stage 1, a 5-minute eyes-closed resting state EEG baseline was recorded under the condition of pink noise as shown in Fig. 1(c). In stage 2, subjects were asked to keep eyes closed and pay attention to 4 sessions of sounds as shown in Fig. 1(d), which may have rhythms. The presentation order of frequencies was counterbalanced across subjects. The BB stimulus lasted for 5 min in each session, and then followed by a 2-minute break. During the first minute of the break, the subjects were allowed to blink their eyes to avoid drowsiness; then they were instructed to stay relaxed with eyes closed again. EEG signals were recorded throughout the whole period.

2.3. Data analysis

The EEG data were preprocessed by removing the ocular artifact and possible interference from head and muscle movements as well as 50 Hz frequency interference. In order to obtain subtle changes, we acquired cluster-corrected permutation statistics based on pair-wised t-tests (Maris and Oostenveld, 2007) to perform the statistical analysis

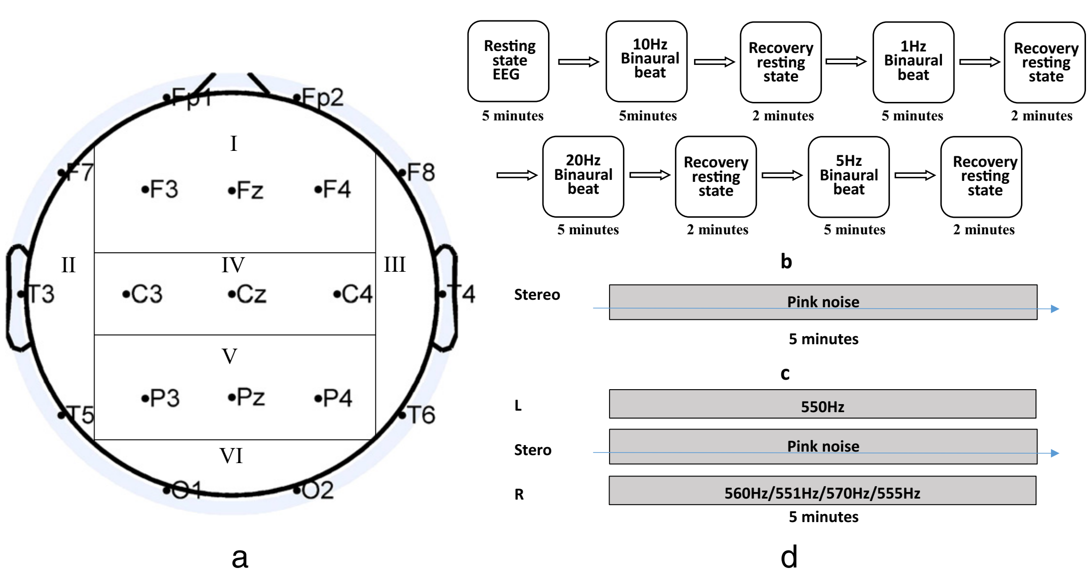


Fig. 1. (a) An illustration of the 19-electrode configuration and the channel groups numbered from I to VI represent the frontal, left temporal, right temporal, central, parietal and occipital cortical regions of the brain respectively. (b) Experimental diagram. (c) Experimental protocol and timings for resting state EEG. (d) Experimental protocol and timings for binaural beat stimulation.

on RP. We clustered the electrodes in connected sets based on spatial adjacency (absolute t-value is larger than 2.16). Then we performed permutation test using the cluster having the largest intra-cluster t-value sum.

The PLV and CMI between the waveforms were calculated for all possible pairs of electrodes (for each electrode, there were $19 \times 18/2 = 171$ possible coherences). Then, we compared repeated-measures ANOVA and simple main effect between the BBs and resting state, where different frequencies and time points were treated as different factors.

Firstly, we analyzed the EEG signals in the minute before each stimulation session (the 5th, 12th, 19th and 26th minute respectively) with the RP, PLV and CMI methods. Statistical analysis showed no significant differences (P-value >0.100) during this 1 minute time period, which indicated that a 2-minute relaxation is sufficient to bring the brain back to a stable resting state. After that we divided each session (5 min) into four sub-sessions (minutes 0.5–1.5, 1.5–2.5, 2.5–3.5 and 3.5–4.5) and then compared the RP, PLV and CMI characteristics on each sub-session between the BB state and the resting state (e.g., RP of EEG data in minute 0.5–1.5 in resting state vs. RP of EEG data in minute 0.5–1.5 in alpha BB).

2.3.1. Relative change of relative power

Power spectral density describes how the power of a signal or time series distributed over different frequencies. Here, EEG power is defined as the squared value of the EEG signal. The total power P of a signal $x(t)$ is given by Eq. (1).

$$P = \lim_{T\to\infty} \frac{1}{2T}\int_{-T}^{T} x(t)^2 \mathrm{d}t \tag{1}$$

$$RP_j = \frac{P_j}{\sum P} \qquad (j = 1, 2, 3, 4) \tag{2}$$

$$RRP_j = \frac{RP_j - RP_{0j}}{RP_{0j}} \qquad (j = 1, 2, 3, 4) \tag{3}$$

Eq. (2) defines relative power RP_j of each band in BBs, where P_j represents the power of frequency band (for $j = 1,2,3,4$, P_j represents the power of delta band (0.5–4 Hz), theta band (4–8 Hz), alpha band (8–12 Hz) and beta band (12–30 Hz), respectively); $\sum P$ is the sum power from 0.5 Hz to 30 Hz. Eq. (3) defines the relative change of relative power RRP_j between BBs and RS, where RP_{0j} is relative power in RS. The positive value of RRP_i means that the relative power is increased after BB, while negative value means decreased.

2.3.2. Phase locking value

By measuring the significance of the phase covariance, the PLV can directly quantify frequency-specific synchronization between two neuroelectric signals (Lachaux et al., 1999). Thus, to examine the role of neural synchronies as a putative mechanism for long-range neural integration in cognitive tasks, the PLV at time t is then defined as shown in Eq. (4).

$$PLV_t = \frac{1}{N}\left|\sum_{n=1}^{N} \exp(j\theta(t,n))\right| \tag{4}$$

where $\theta(t, n)$ is the phase difference $\varphi_1(t, n) - \varphi_2(t, n)$.

PLV measures the inter-trial variability of phase difference at time t: If the phase difference varies little across the electrodes, PLV is close to 1; otherwise it is close to zero. This procedure can be repeated for several frequencies in order to study a broader frequency range.

2.3.3. Cross-mutual information

Measuring the brain network connectivity by cross-mutual information (CMI) is an effective way to study the relationship between different regions of the brain (Jeong et al., 2001). In probability and information theory, the mutual information of two random variables is the quantitative measurement of the mutual dependence of the two random variables. The most common unit of measurement of mutual information is bit, where logarithm to the base 2 is used. Generally, the mutual information of two continuous random variables X and Y can be defined as Eq. (5):

$$I(X;Y) = \int_Y \int_X p(x,y)\, \log\left(\frac{p(x,y)}{p(x)p(y)}\right) dxdy \tag{5}$$

where $p(x, y)$ is the joint probability density function of X and Y; $p(x)$ and $p(y)$ are the marginal probability density functions of X and Y, respectively.

Electrodes placed at different brain regions constructed a brain network. The mutual information between EEG signals from electrodes X and Y demonstrates the similarity of the corresponding brain regions. In another word, it measures the degree of uncertainty reduction of one variable with a known one. The larger the value $I(X;Y)$, the higher the connectivity between these two brain regions.

3. Results

3.1. Relative power analysis

The relative power (RP) of four frequency bands was calculated and analyzed by cluster-corrected permutation statistics based on pair-wised t-tests. After RRP calculation, we obtained the topographical images of different bands at different time periods. Fig. 2 shows the results under delta BB, theta BB and beta BB, and Fig. 3 shows the results under alpha BB. The RP values and other details of electrodes were shown in Table 1. For better comparison, we presented the power spectral density together with the RRP topographical images, which showed the rate of increase or decrease of RP. In the topographical images, red color indicated increased RP after BB stimulus while blue decreased.

3.1.1. RRP in delta BB

As shown in Fig. 2, during delta BB (1 Hz), the RP of theta band on F7 decreased in minute 0.5–1.5. In the next minute, the RP of alpha band on T4 decreased significantly. Meanwhile, the RP of beta band increased in C3, T5, P3, Pz and P4. However, all changes vanished after 2.5 min, and no change was observed in delta band throughout the period.

3.1.2. RRP in theta BB

During theta BB (5 Hz), only the RP of beta band on T3 reduced in minute 1.5–2.5, as shown in Fig. 2. No significant change was observed in other bands or other time intervals.

3.1.3. RRP in alpha BB

As shown in Fig. 3, during alpha BB (10 Hz), firstly (minute 0.5–1.5) the RP of theta band on F8 was decreased, while the RP of beta band on C3 and Cz was increased. Then, in minute 1.5–2.5, the RP increased in delta band, but decreased in theta and alpha bands on left temporal cortical area. In the next minute (2.5–3.5), the RP kept increasing or decreasing in different frequency bands but the position changed. For delta and alpha bands, the trigger position changed from left temporal area to the right temporal cortical area; for theta band, the RP decreased almost in whole brain. Meanwhile, the RP of beta band increased on P4. Still, no change presented in the last minute.

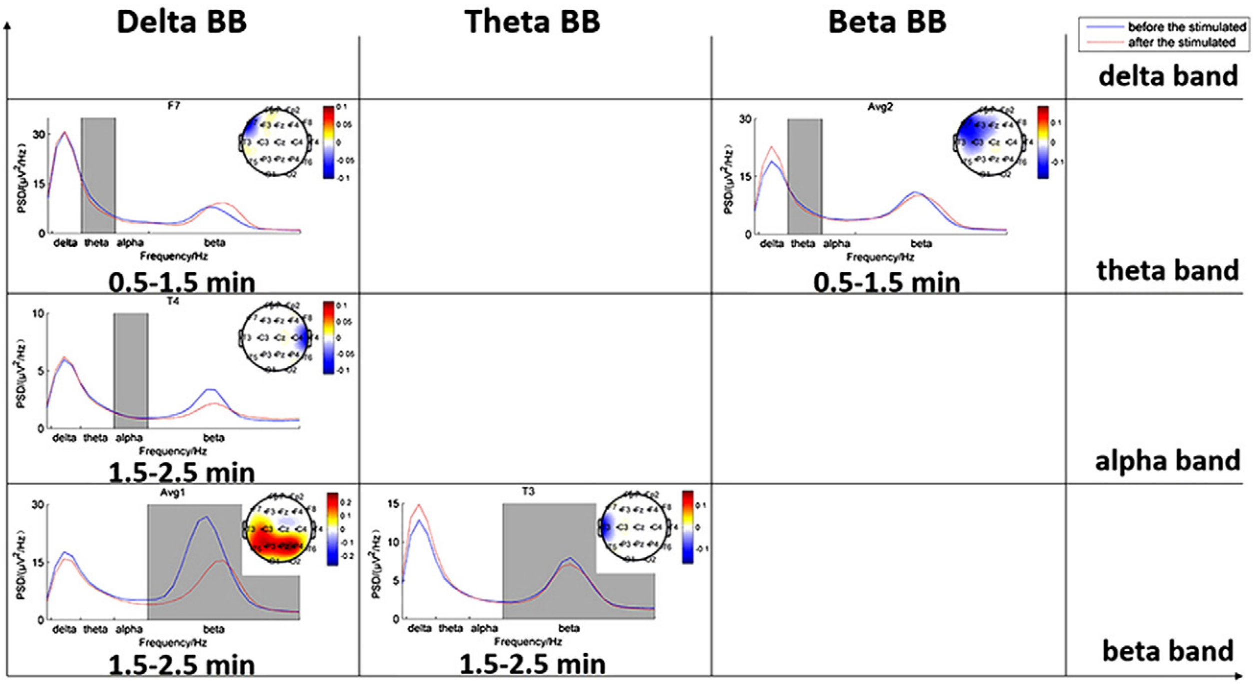


Fig. 2. Power spectral density and RRP topographical images in delta, theta and beta BBs. The first row is that of delta BB; the second row is that of theta BB; the third row is that of beta BB. Line 1 to line 4 indicate delta, theta, alpha and beta bands in EEG. Among them, significant cluster ($P < 0.05$) is the RRP between BBs and resting state. In topographical images, the red areas represent the decreased RP after BB stimulus while the blue areas represent the decreased RP. Avg1 is the average power spectral density of C3, T5, P3, Pz and P4 electrodes. Avg 2 is the average power spectral density of F7, F3, Fz and C3 electrodes.

3.1.4. RRP in beta BB

As shown in Fig. 2, we noticed that the RP of theta band decreased on F7, F3, Fz and C3 in minute 0.5–1.5 during beta BB (20 Hz) stimulation.

3.2. Phase-locking analysis

By phase-locking analysis, we compared the coherence changes of the brain between resting state and BB stimulations. As shown in

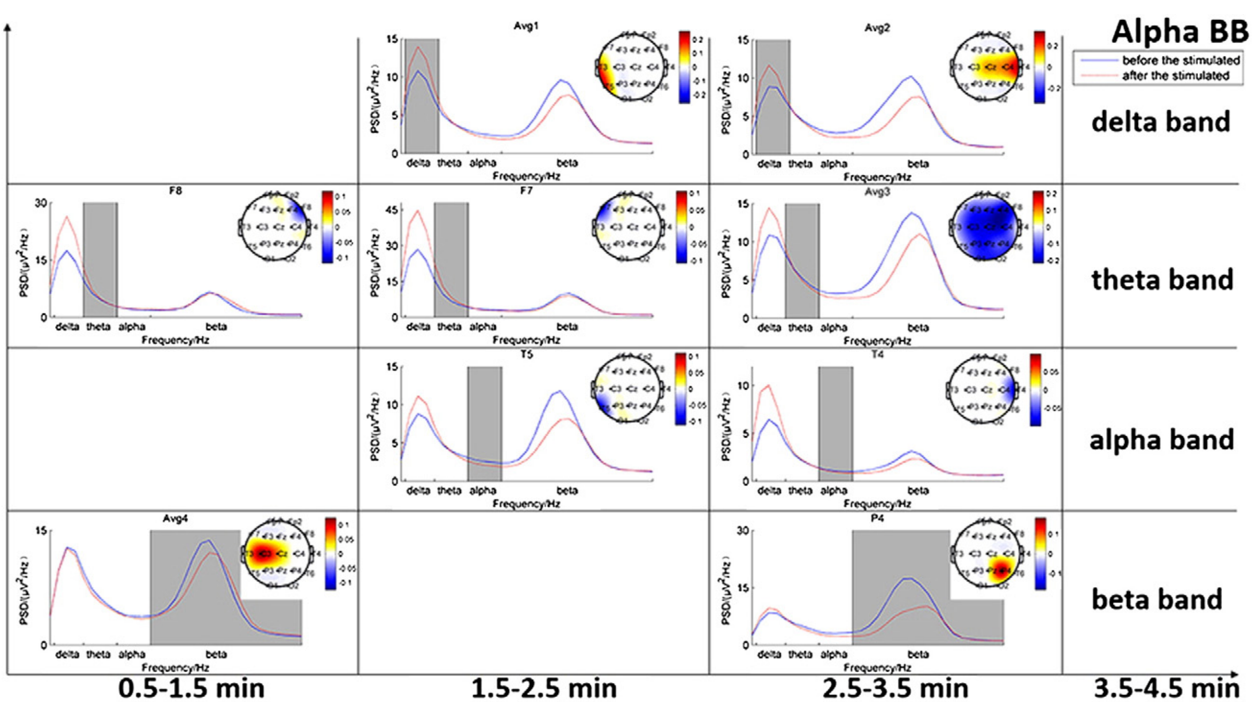


Fig. 3. Power spectral density and RRP in alpha BB ($P < 0.05$). Line 1 to line 4 indicate delta, theta, alpha and beta bands in EEG. Avg1 is the average power spectral density of T3 and T5 electrodes. Avg2 is the average power spectral density of Cz, C4 and T4 electrodes. Avg3 is the average power spectral density of Fz, F4, F8, C3, Cz, C4, P3, Pz, P4, T6, O1 and O2 electrodes. Avg5 is the average power spectral density of C3 and Cz electrodes.

Table 1
Means (SD) and *P*-value for RP and RRP of delta band (0.5–4 Hz), theta band (4–8 Hz), alpha band (8–12 Hz) and beta band (12–30 Hz) in delta BB (1 Hz), theta BB (5 Hz), alpha BB (10 Hz) and beta BB (20 Hz) during four time periods.

BB	Electrode	RP in RS	RP in BB	RRP	*P*-value	Frequency band	Time
Delta	F7	0.153(0.046)	0.136(0.037)	−0.100	0.040	Theta	0.5–1.5
Delta	T4	0.215(0.124)	0.163(0.071)	−0.109	0.046	Alpha	1.5–2.5
Delta	C3	0.202(0.056)	0.232(0.066)	0.166	0.020	Beta	1.5–2.5
Delta	T5	0.224(0.063)	0.255(0.072)	0.154	0.020	Beta	1.5–2.5
Delta	P3	0.200(0.062)	0.236(0.067)	0.224	0.020	Beta	1.5–2.5
Delta	Pz	0.182(0.061)	0.211(0.063)	0.233	0.020	Beta	1.5–2.5
Delta	P4	0.196(0.070)	0.233(0.072)	0.266	0.020	Beta	1.5–2.5
Theta	T3	0.303(0.111)	0.248(0.106)	−0.164	0.037	Beta	1.5–2.5
Alpha	F8	0.148(0.048)	0.132(0.052)	−0.116	0.020	Theta	0.5–1.5
Alpha	C3	0.195(0.049)	0.220(0.064)	0.125	0.004	Beta	0.5–1.5
Alpha	Cz	0.165(0.044)	0.177(0.054)	0.067	0.004	Beta	0.5–1.5
Alpha	T3	0.362(0.117)	0.429(0.168)	0.189	0.021	Delta	1.5–2.5
Alpha	T5	0.321(0.127)	0.376(0.134)	0.242	0.021	Delta	1.5–2.5
Alpha	F7	0.139(0.047)	0.123(0.043)	−0.106	0.040	Theta	1.5–2.5
Alpha	T5	0.358(0.166)	0.310(0.137)	−0.111	0.020	Alpha	1.5–2.5
Alpha	Cz	0.332(0.133)	0.375(0.156)	0.151	0.021	Delta	2.5–3.5
Alpha	C4	0.334(0.126)	0.382(0.154)	0.172	0.021	Delta	2.5–3.5
Alpha	T4	0.373(0.142)	0.473(0.172)	0.338	0.021	Delta	2.5–3.5
Alpha	F3	0.193(0.060)	0.165(0.058)	−0.129	0.003	Theta	2.5–3.5
Alpha	Fz	0.222(0.068)	0.186(0.062)	−0.142	0.003	Theta	2.5–3.5
Alpha	F4	0.211(0.063)	0.167(0.052)	−0.191	0.003	Theta	2.5–3.5
Alpha	F8	0.154(0.051)	0.122(0.044)	−0.173	0.003	Theta	2.5–3.5
Alpha	C3	0.189(0.056)	0.156(0.048)	−0.152	0.003	Theta	2.5–3.5
Alpha	Cz	0.227(0.071)	0.181(0.054)	−0.181	0.003	Theta	2.5–3.5
Alpha	C4	0.212(0.066)	0.164(0.044)	−0.195	0.003	Theta	2.5–3.5
Alpha	P3	0.187(0.056)	0.158(0.040)	−0.121	0.003	Theta	2.5–3.5
Alpha	Pz	0.193(0.060)	0.155(0.041)	−0.162	0.003	Theta	2.5–3.5
Alpha	P4	0.182(0.057)	0.153(0.040)	−0.111	0.003	Theta	2.5–3.5
Alpha	T6	0.145(0.056)	0.120(0.045)	−0.141	0.003	Theta	2.5–3.5
Alpha	O1	0.160(0.053)	0.136(0.046)	−0.125	0.003	Theta	2.5–3.5
Alpha	O2	0.141(0.051)	0.115(0.039)	−0.152	0.003	Theta	2.5–3.5
Alpha	T4	0.204(0.108)	0.162(0.087)	−0.097	0.046	Alpha	2.5–3.5
Alpha	P4	0.189(0.067)	0.217(0.081)	0.184	0.022	Beta	2.5–3.5
Beta	F7	0.153(0.046)	0.124(0.046)	−0.185	0.007	Theta	0.5–1.5
Beta	F3	0.191(0.059)	0.171(0.061)	−0.110	0.007	Theta	0.5–2.5
Beta	Fz	0.207(0.066)	0.194(0.068)	−0.064	0.007	Theta	0.5–2.5
Beta	C3	0.184(0.056)	0.168(0.063)	−0.098	0.007	Theta	0.5–2.5

Fig. 4, red lines between electrodes represent phase-locking increasing after stimulation. After calculated phase-locking of each electrode, we analyzed the values by repeated-measures ANOVA. It can be seen that in BBs there was no significant change in any frequency bands before the last minute. However, the PLV of theta band increased in the last minute during all but theta BB (Fp1-T6, Fp1-O2, F7-T6, F4-O2 and F7-O2 in delta BB; Fp1-O2 in alpha BB and Fp1-O2 and F3-T6 in beta BB). Means and *P*-values for them were shown next to the topographies in Fig. 4.

3.3. Cross-mutual information analysis

Using CMI, we compared the information transmission changes of the brain between during resting state and during four types of stimulations. As shown in Table 2 and Fig. 5, red lines between electrodes represent CMI increasing after stimulations, while blue ones represent decreasing.

3.3.1. CMI in delta BB

There was no significant information of connectivity changes in first 2.5 min and in the last minute. However, in minute 2.5–3.5, the CMI declined in temporal and occipital area (between F8-O1 and F8-T6).

3.3.2. CMI in theta BB

In the first minute, the CMI decreased mainly between left temporal and other cortical areas (between C4-T3, C4-P3 and C4-Pz). In minute 2.5–3.5, increased CMI was mainly observed in left hemisphere (Fp1-C4, Fp2-T3, Fp2-C3, Fp2-P3, F3-T3, Fz-T3, T3-C4 and Fz-T4). No changes presented in the last minute.

3.3.3. CMI in alpha BB

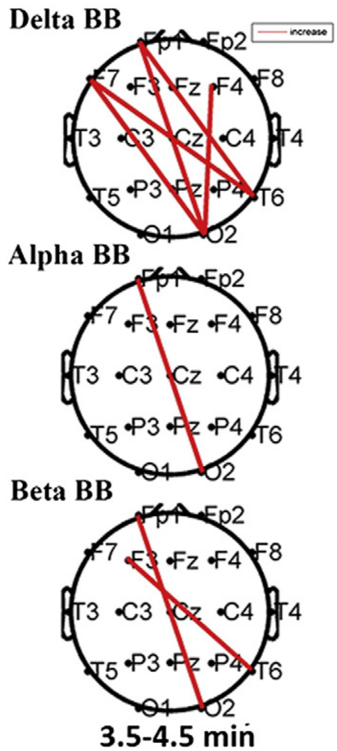
During alpha BB stimulation, before the first 2.5 min and in the last minute, there was no significant change of CMI. In minute 2.5–3.5, the CMI decreased in right temporal (between Fz and T4).

3.3.4. CMI in beta BB

We noted that, in the first minute, the CMI enhanced between left temporal and frontal cortical area (between F7-T3 and T3-F3), while, decreased in right temporal area (between F7-T3, T3-F3, Cz-C4, C4-T4, C4-P3, C4-Pz, T4-Pz and T4-P4). In the following minute, the CMI increased between F7-T3 and F7-T4. No change was observed in minute 2.5–3.5. In the last minute, the CMI of right hemisphere increased (between Fp2-Pz and Fp2-T6).

4. Discussion and conclusion

When exposed to 5-minute BBs (delta (1 Hz), theta (5 Hz), alpha (10 Hz) and beta (20 Hz)), the relative power of dominant EEG frequency of 13 subjects showed no enhancement. Our results didn't support the brainwave entrainment effect or FFR hypothesis that the human brain had a tendency to change its dominant EEG frequency under BB stimulations. Different to previous work (Stevens et al., 2003; Wahbeh et al., 2007; Goodin et al., 2012; Vernon et al., 2012), we extended the duration of BB stimulations to 5 min with 4 modulation frequencies, which allows us to compare the results between different BBs. However, by tracking the changes of relative power (RP) instead of power, we discovered several interesting phenomena. In delta and alpha BBs, RP of theta and alpha bands decreased, while RP of beta band increased.



Means (SD) and P value for PLV of theta band (4-8 Hz) in delta BB (1 Hz)

BB	Electrode	PLV in RS	PLV in BB	P-value	Frequency band	Time
delta	Fp1-T6	0.212(0.045)	0.267(0.071)	0.025	theta	3.5-4.5
delta	Fp1-O2	0.183(0.030)	0.240(0.058)	0.004	theta	3.5-4.5
delta	F7-T6	0.212(0.036)	0.267(0.066)	0.014	theta	3.5-4.5
delta	F4-O2	0.205(0.059)	0248(0.067)	0.004	theta	3.5-4.5
delta	F7-O2	0.195(0.035)	0.252(0.054)	0.004	theta	3.5-4.5

Means (SD) and P value for PLV of theta band (4-8 Hz) in alpha BB (10 Hz)

BB	Electrode	PLV in RS	PLV in BB	P-value	Frequency band	Time
alpha	Fp1-O2	0.183(0.030)	0.235(0.053)	0.005	theta	3.5-4.5

Means (SD) and P value for PLV of theta band (4-8 Hz) in beta BB (20 Hz)

BB	Electrode	PLV in RS	PLV in BB	P-value	Frequency band	Time
beta	Fp1-O2	0.239(0.053)	0.313(0.113)	0.004	theta	3.5-4.5
beta	F3-T6	0.183(0.030)	0.255(0.074)	0.043	theta	3.5-4.5

Fig. 4. The PLV of delta band in delta, alpha and beta BBs during minute 3.5–4.5. Red lines between electrodes mean increasing after stimulus. Means and *P*-values for significant changes were shown besides topographies.

Previous studies showed that increased theta activity may indicate early stage of drowsiness. Besides, it has been reported that train drivers with increased alpha activity may fall asleep while driving (Åkerstedt and Gillberg, 1990; Torsvall and åAkerstedt, 1987). It has also been presented that alpha activity may be associated with auditory attention allocation (Foxe and Snyder, 2011). Furthermore, beta EEG activity has been reported to associate with attention dysfunction (Lubar et al., 1995a,b) and alertness level (Eoh et al., 2005). Moreover, enhanced beta power in EEG may lead to improved mental focuses in both patients (Fuchs et al., 2003; Monastra et al., 2006) and healthy controls (Rasey et al., 1996; Egner and Gruzelier, 2004).Our studied showed that delta and alpha BBs can lead to decreased theta and alpha activity and increased beta activity. This may suggest an approach to reduce drowsiness and improve mental focus, which in turn can treat attention-deficit hyperactivity disorder (Barry et al., 2003; Leins et al., 2007). On the other hand, Schizophrenia and Depression patients present increased EEG power in beta band (Tekell et al., 2005; Yeragani et al., 2006). Therefore, introduced RP decrease in beta band by using theta BB may help in treating Schizophrenia and Depression. Fig. 3 also showed that RP in delta band increased under alpha BB stimulation. However, this may be caused by EEG signal acquisition process rather than BB induced effect. It has been reported that short-lasting painful stimuli could lead to delta activity increase (Bromm et al., 1989; Chang et al., 2002). Similarly, uncomfortable scalp electrodes with conductive paste may also lead to enhance delta activity of the subjects.

Table 2
Means (SD) and *P*-value for CMI of delta band (0.5–4 Hz), theta band (4–8 Hz), alpha band (8–12 Hz) and beta band (12–30 Hz) in delta BB (1 Hz), theta BB (5 Hz), alpha BB (10 Hz) and beta BB (20 Hz) during four time periods.

BB	Electrode	CMI in RS	CMI in BB	*P*-value	Time
Delta	F8-O1	0.197(0.085)	0.120(0.093)	0.046	2.5–3.5
Delta	F8-T6	0.227(0.063)	0.159(0.091)	0.044	2.5–3.5
Theta	T3-C4	0.286(0.106)	0.207(0.067)	0.037	0.5–1.5
Theta	C4-P3	0.410(0.129)	0.312(0.099)	0.048	0.5–1.5
Theta	C4-Pz	0.532(0.103)	0.434(0.112)	0.036	0.5–1.5
Theta	Fp1-C4	0.245(0.081)	0.333(0.116)	0.041	2.5–3.5
Theta	Fp2-T3	0.177(0.122)	0.289(0.111)	0.027	2.5–3.5
Theta	Fp2-C3	0.257(0.102)	0.363(0.111)	0.023	2.5–3.5
Theta	Fp2-P3	0.168(0.113)	0.264(0.114)	0.047	2.5–3.5
Theta	F3-T3	0.372(0.139)	0.482(0.107)	0.038	2.5–3.5
Theta	Fz-T3	0.269(0.099)	0.360(0.089)	0.026	2.5–3.5
Theta	T3-C4	0.208(0.074)	0.301(0.104)	0.018	2.5–3.5
Alpha	Fz-T4	0.287(0.089)	0.281(0.074)	0.018	2.5–3.5
Beta	F7-T3	0.406(0.132)	0.541(0.154)	0.030	0.5–1.5
Beta	T3-F3	0.376(0.120)	0.477(0.116)	0.046	0.5–1.5
Beta	Cz-C4	0.733(0.137)	0.612(0.115)	0.027	0.5–1.5
Beta	C4-T4	0.408(0.115)	0.298(0.091)	0.016	0.5–1.5
Beta	C4-P3	0.410(0.129)	0.305(0.102)	0.037	0.5–1.5
Beta	C4-Pz	0.532(0.103)	0.398(0.108)	0.005	0.5–1.5
Beta	T4-Pz	0.288(0.095)	0.188(0.080)	0.010	0.5–1.5
Beta	T4-P4	0.326(0.112)	0.240(0.074)	0.036	0.5–1.5
Beta	F7-T3	0.398(0.133)	0.562(0.166)	0.013	1.5–2.5
Beta	F7-T4	0.142(0.069)	0.213(0.081)	0.032	1.5–2.5
Beta	Fp2-Pz	0.166(0.102)	0.263(0.094)	0.024	3.5–4.5
Beta	Fp2-T6	0.110(0.073)	0.194(0.080)	0.015	3.5–4.5

Besides brainwave oscillation, functional brain connectivity changes induced by BB stimulations may provide new insight into the understanding of the mechanism of BB induced behavior and cognition changes. In this study, we employed two approaches, phase locking value (PLV) and cross-mutual information (CMI), to measure the brain connectivity variations before and during BB stimulations. By measuring the significance of the phase covariance between two signals, PLV reflected frequency-specific synchronization between two neuro-electric signals. Under delta, alpha and beta BBs, increased anterior–posterior intra-cerebral connectivity in the theta band was observed. Lehmann et al. (2006) reported similar results that, during meditation, PLV between anterior and posterior area was increased in the theta band. Therefore, the 5-minute BBs induced phase locking change may help to deepen relaxation meditation. Moreover, RP of

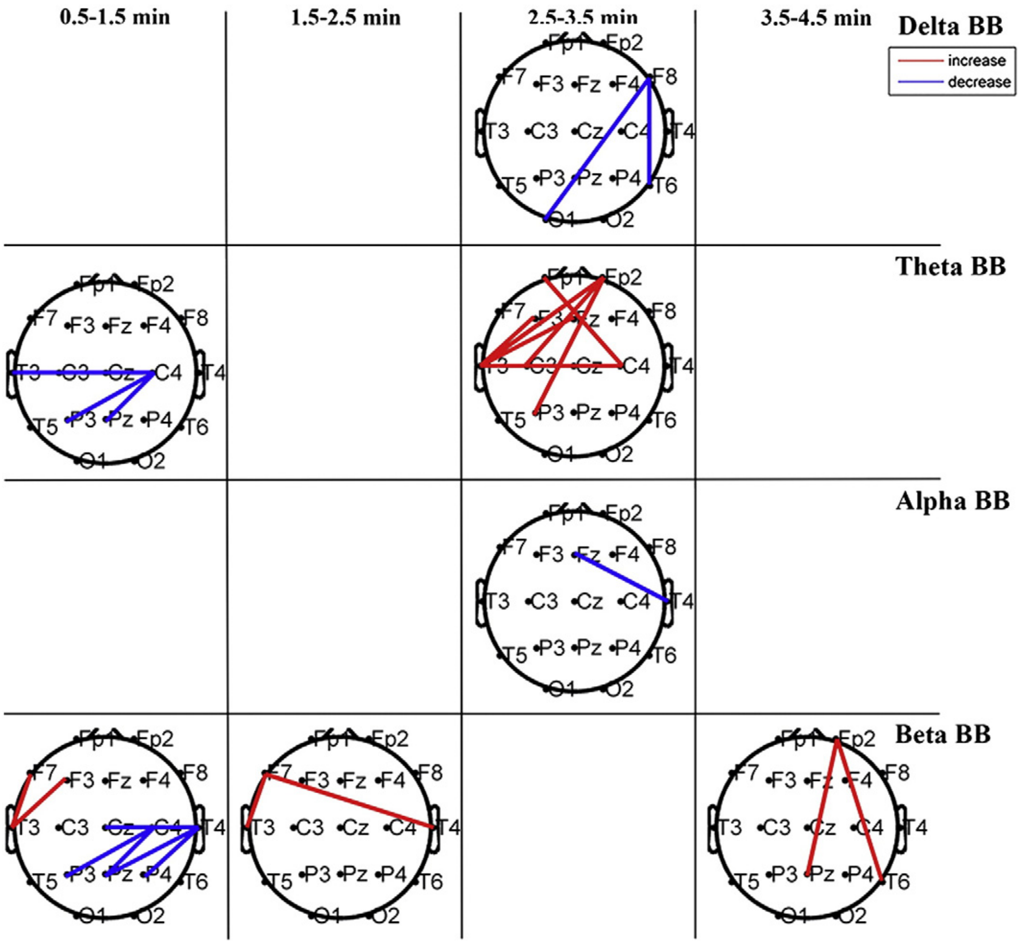


Fig. 5. CMI in BBs. Line 1 to line 4 indicate CMI in delta, theta, alpha and beta BBs during four time periods. Red lines represent increased CMI after stimulus, and the blue ones represent decreased CMI.

theta band was decreased during BBs (delta, theta and beta BBs) before the last minute, and then the intra-cerebral PLV of theta band increased in anterior–posterior area during the last minute. Our findings may suggest that, with the time going during the simulation, coherence variation will dominate the EEG signal changes rather than RP, which may help to explain the mechanism of physiological and psychological alterations during BB stimulation. Phase locking of theta band was involved in many other physiological processes, such as working memory and episodic memories (Lee et al., 2005; Summerfield and Mangels, 2005). However, it is less clear why there is no PLV change in other bands. Therefore, further work is needed to study the relation among relative power, coherence and BBs.

Another connectivity measurement between different cortical areas is CMI, which measures dynamical coupling or information transmission between two time series. Notably, in this study, we observed different CMI variations under different BB stimulations. During delta and alpha BB stimulations, the CMI reduced among right temporal, frontal and occipital cortical area in minute 3.5. Some studies reported that the schizophrenia patients had significantly higher intra-hemispheric coherence compared to controls (Merrin et al., 1989; Wada et al., 1998; Na et al., 2002). Therefore, the delta and alpha BBs may be used as an assistant treatment of schizophrenia. Under theta and beta BBs, CMI decreased at first and then increased. This information transmission enhancement by BB stimulation may be a possible approach to increase hypnotic susceptibility (Stevens et al., 2003).

It was also interesting to point out that, the brain locations showing changes were different under different BB stimulations. This indicated that the left and right temporal cortical areas respond differently to BB stimulations. Moreover, the changes not only showed in temporal area, core area of auditory system, but also in central and other areas. This may suggest that the top-down control is involved (Gilbert and Sigman, 2007). In addition, the responses in RP and CMI were discontinuous. Some of the observed measurement changes appeared at first and then vanished during the time of stimulations. This may be caused by the insufficient sound pressure level or the distraction of the subjects. It is worth mentioning that the modulated frequencies are 1, 5, 10, and 20 Hz. Further studies with BBs of different modulated frequencies should be tested to validate the observations of this work.

To sum up, we observed no significant brainwave entrainment effect or Frequency Follow Response under delta (1 Hz), theta (5 Hz) alpha (10 Hz) and beta (20 Hz) BB stimulations in RP analysis. However, delta and alpha BBs could provoke RP increase in theta and alpha bands and RP decrease in beta band. Theta BB could induce decreased RP in beta band, while beta BB induced decreased RP in theta band. In PLV analysis, increased intra-cerebral brain functional connectivity between anterior and posterior area in the theta band was detected only under delta, alpha and theta BBs situations. However, when using cross-mutual information, we observed decreased connectivity in delta and alpha BBs, and decreased at first and then increased in theta and beta BBs. Our results support the hypothesis that binaural beats can induce brain connectivity changes.

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