Title: Alteration of Brain Functional Network and Cortisol Level During Induction and Release of Stress: An EEG Study in Young Male Adults

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Abstract

Acute stress in a long period of time could drastically influence one's behavioral and cognitive performances. Therefore, it is important to control the stressful situation and release it after a stressful event. In this regard, understanding of brain mechanism of the stress release will help to introduce new practical approaches. In this study, we hypothesized that induction and release of stress will change the brain functional connectivity pattern. Therefore, by recruiting 20 healthy-subjects and exposing them to stressful events using the Trier Social Stress paradigm, we aimed to investigate patterns of these changes. In a session consist of 23 minutes of psychological stress induction and 20 minutes of recovery, subjects' stress was scored by visual analogue scale (VAS). In addition, salivary cortisol level and EEG data of the subjects were also recorded. Subsequently, brain functional connectivity (FC) maps were calculated in a frequency-specific manner. Then, the effects of inducing and release of stress on the changes of VAS, cortisol level, and FC maps were assessed. Our results revealed that inter-hemispheric FCs of the right frontal regions with other regions of the brain decrease; while it increases at the left frontal regions during inducing of mental stress. Interestingly, therelease of stress presented a recovery pattern of inter-hemispheric FCs and meaningful FC changes significantly correlate with changes in the cortisol level. our findings highlight important roles of bihemispheric associations in adaptation and coping with stressful conditions.

Keywords: Stress, Visual analogue scale, Cortisol, EEG, Functional connectivity.

Introduction

The behavioral and cognitive performances could be crucially influenced by stressful events in every day. Stress is defined as a behavioral response to uncertain conditions in which the proper responses of body have appeared in the form of chemical adjustment and physiological changes (Peters, McEwen et al. 2017). These biological modifiers should have enough time to restore the normal state of homeostasis, otherwise, the chronic stress would be anticipated (McEwen 2007) which is an aggregator for many disorders (Yaribeygi, Panahi et al. 2017).

Previous studies have reported two neuroendocrine systems for responding to stressful events including activation of hypothalamus- pituitary- adrenal axis that regulates release of the glucocorticoid (GC) hormones (mainly cortisol in humans)_, and sympathetic- adrenal-medullary system that increases the sympathetic tone (Yaribeygi, Panahi et al. 2017). The GC has many receptors in the brain that following a stressful condition could change the brain activities (McEwen, Bowles et al. 2015). The changes in brain activities could occur in the form of changes in neural oscillations. (Putman, Verkuil et al. 2014) (Hamid, Sulaiman et al. 2010) (Lewis, Weekes et al. 2007). While the pattern of neural oscillations could be traced by measuring EEG signal in a range of millisecond. Based on the EEG signal, interactions between pairs of electrodes could also be calculated (Schoffelen and Gross 2009, Khosrowabadi 2018) (Achard, Salvador et al. 2006).

These interactions between various brain regions are crucial for normal brain functioning that could be measured in the form of synchrony between them and are called functional connectivity (FC) (Achard, Salvador et al. 2006) (Garmezy and Rutter 1983). The FC approach has been implied for the study of stress (Khosrowabadi, Quek et al. 2011, Khosrowabadi 2018)

(Alonso, Romero et al. 2015). These studies have reported an inverted-u-shaped relationship between GC levels and cognitive performance in stressful condition (Lupien, McEwen et al. 2009). In acute stress condition, the GC receptors are activated which destabilized the already established synaptic connection (Hüther 1998). Subsequently, neural pathway associated between cortical and limbic system is facilitated to trigger large-scale brain networks (Hermans, Henckens et al. 2014). For instance, the neural system handled by salience processing network is triggered and facilitated the relocation of executive-control-network (Hermans, Henckens et al. 2014). This effect follows in a BiHemispheric autonomic model (Tegeler, Shaltout et al. 2015). The BHAM model relates the right hemisphere to the sympathetic response 'fight or flight', and the left hemisphere responsible to the parasympathetic response 'rest, digest or freeze'. Nevertheless, the mechanism of changes in the FC network ofthe brain during induction and release of stress still need to be well understood. Therefore, in this study, we used a whole-brain approach using EEG data to investigate changes in FC network while exposed to acute stressor and after recovery. We aimed to test the BHAM model on these data. The hypothesis was stress change the FC of brain activity based on EEG and after 20 minutes of recovery the changes return to pre-stress. Therefore, 20 male-subjects were recruited and their psychological stress scores, salivary cortisol level, and EEG data were recorded before and after stress induction and after 20 minutes of recovery. Then, behavioral, physiological and neurophysiological markers at three mentioned conditions were statistically compared.

Materials and Methods

Participants

Twenty healthy male subjects with mean (SD) age of 23.37±2.7 were recruited from students of Baqiyatallah university of medical science for the study. All the participants are right-handed. The inclusion criteria consisted of general physical and mental health, no smoking habit, no surgery in the spine and cervicocephalic, no regular neuropsychological medication usage, no regular exercise (minimally three times in week), and no abnormal sleep pattern. All participants signed a consent form approved by Baqiyatallah University of Medical Science ethical board before the experiment.

Experimental design

The experiment consisted of two phases including 23 minutes of stress induction and 20 minutes of recovery and three measurements (pre-stress, post- stress and post- recovery) . The subjects' psychological stress scores were measured by visual analogue scale (VAS) (Hellhammer and Schubert 2012). Also, their salivary cortisol level and EEG data were also recorded. The stress was induced by trier social stress test (TSST) (Kirschbaum, Pirke et al. 1993) and recordings were performed before and after stress induction and after the recovery phase.

Trier social stress test

TSST is a standardized protocol for the generation of moderate psychosocial stress in laboratory settings (Kirschbaum, Pirke et al. 1993). The TSST consists of 3 minutes brief preparation period followed by 10 minutes of test period in which the subject has to deliver a free speech for 2 minutes and perform a mental arithmetic task for 8 minutes in standing position in front of two referees behind the desk. During his speech, referees with neutral faces only listened to him and warned him to continue when he stopped talking. After the first 2 minutes, the participant was asked to count down from 1022 to 13, and at each wrong subtraction, he was warned to start counting down from the beginning. The perceived stress and anxiety score were measured by VAS questionnaire that measured the score of self-reporting of stress before and after test and after 20 minutes of recovery.

Salivary cortisol level

Following the activation of the Hypothalamic–pituitary–adrenal axis and Sympathomedullary Pathway (Baum and Contrada 2010), cortisol is released from the adrenal gland into the bloodstream and spreads throughout the body. Changes in cortisol level have been introduced as a standard stress index that could be measured using the blood or salivary test (Zigmond and Bloom 1999, Dickerson and Kemeny 2004). In this study, a salivary cortisol test was performed to confirm the results of TSST. The subjects were asked to eat nothing an hour before the test and wash their mouth right before the test. An ELISA kit of IBL Company, made in Germany, was used in the following procedure. 0.5 ml of salivary sample was gathered before and after stress induction and after 20 minutes of recovery; then the samples were frozen at -80°C. The salivary cortisol levels were then measured from the frozen samples. A statistical analysis was then performed to compare data of the 3 conditions.

EEG data acquisition

EEG data were recorded using a 32 channel amplifier (Mitsar Co Ltd, EEG 202) positioned according to the standard international 10-20 system (one minute with closed eyes and one minute with open eyes). An EEG cap with 32 reusable sintered Ag/AgCl electrode was applied and the scalp skin beneath each electrode was kept clean by slight abrasion and cleaning with alcohol. An impedance check was performed and resistances below 10 k ohm accepted. A bandpass of 0.1-70Hz was considered during the recording and the EEG data were recorded using a 12-bit digitizer with sampling frequency of 256 Hz.

EEG data processing and analysis

A standard preprocessing was performed on the EEG data using the Matlab EEGlab toolbox. The preprocessing was consisted of the following sessions including conversion of the EEG data format readable in Matlab 2017b, filtering unwanted noises using a bandpass FIR filter from 1-40 Hz, Epoching the data to segments of 1 second. employing ADJUST plugin to remove artifacts based on ICA., interpolation of bad channels detected by kurtosis of EEG data. Rereference all the electrodes of the average of all channels. After preprocessing, functional connectivity (FC) is considered as the temporal dependency between neuronal activations of pairs of electrodes (Lang, Tomé et al. 2012). The FC network was estimated by taking partial correlation between pairs of electrodes (Friston 1994, Lang, Tomé et al. 2012). In the context of brain networks, partial correlation is the correlation between time series of two nodes, after adjusting for the time series from all other network nodes as covariate factors (Wang, Kang et al. 2016). Functional connectivity analysis was performed on the conventional frequency bands including delta [1-4 Hz], theta [4-8 Hz], alpha [8-13 Hz], beta [13-30 Hz], and lower-gamma [30-40 Hz].

Statistical analysis

Statistical analysis was performed on the visual analogue scale, salivary cortisol and FC maps measured before stress induction, after 23 minutes of inducing mental stress and 20 minutes after the recovery. After test of normality by the Kolmogorov Smirnov test, the statistical analysis was performed using two distinct paired t-test for stress induction phase and recovery phase. Firstly, a paired t-test was applied between data gathered before, and after stress, induction to identify how stress induction changes the visual analogue scale, salivary cortisol level and pattern of brain connectivity. Subsequently, correlation between relative changes in FC [for instance ($FC_{after\ stress}$-FC_{before})/ FC_{before}] and relative changes in Cortisol [for instance ($Cortisol_{after\ stress}$-$Cortisol_{before}$)/ $Cortisol_{before}$] and VAS [for instance ($VAS_{after\ stress}$-VAS_{before})/ VAS_{before}] was also computed on the data gathered after inducing stress, and after recovery. Relative changes are calculated based on difference between two conditions divided by the value of initial condition. Finally, the statistical results were corrected for multiple comparisons effect using the false discovery rate (FDR) (Benjamini and Hochberg 1995) (Shaffer 1995).

Results

Behavioral and physiological changes

The average changes in the visual analogue scales in three measurements are presented in Figure 2. The results showed that the VAS scores significantly increased by stress induction. As expected, the VAS scores also significantly decreased after the release of stressful condition (pre-stress: 1.1±1, post-stress: . 3.2±2, post-recovery: 0.6±0.7) (F=22.91, p<0.0000 for significant different between post-stress and pre-stress and post-recovery)

The salivary cortisol levels at three stages of the experiment (pre-stress: 2.5±1 , post-stress: 4.4±2 , post-recovery: 5.1±4 μm/dl) are also presented in Figure 3. The results showed a significant increase of cortisol after induction of mental stress ($p<0.017$). Although, the cortisol level still increased in the recovery phase alteration was not significant after the recovery.

Alteration of brain functional connectivity

As it was described in the previous section, the subject-wise FC pattern was calculated based on synchrony between EEG signals of pair of electrodes using partial correlation approach (Baba, Shibata et al. 2004). Subsequently, statistical comparison was performed using the paired t-test. A correction for multiple-comparison was then performed that the significant changes are presented in Figure 4.

The results showed that stress induction mostly changes the fronto-temporal functional connections especially in delta, alpha, beta and gamma bands ($P<0.05$, FDR corrected) mainly observed in the right hemisphere (see Figure4). In addition, 20 minutes of recovery causes a significant change at the tempro-parietal functional connections especially in theta and beta bands ($P<0.05$, FDR corrected). In the recovery phase, FCs of the right frontal region increased while FCs in the right parietal region decreased in theta band; in addition, FCs of the left parietal region also increased in the beta band. Also, the most significant results ($P<0.05$, FWE-family-wise error corrected using Bonferroni method) are presented in Table 1.

Association between alteration of brain FCs and cortisol level and VAS

Relative changes of brain FC after stress induction and 20 minutes after release of stress showed a positive correlation with the related changes of cortisol after stress induction. Changes of FC between CP4 and T6 in alpha band ($r=0.75$, $p=0.01$) showed positive correlation with changes in cortisol level (Figure 5A). In addition, changes of FC between FT8 and CPZ in alpha band ($r=-0.65$, $p=0.04$) also showed negative correlation with changes in cortisol level (Figure 5B).

Discussion

The aim of our study was to evaluate the effect of short-term psychological stress and its release on the brain functional connectivity. Our findings demonstrate that stressful events change the fronto-temporal connections, especially in delta, alpha, beta and gamma bands. Moreover, inter-hemispheric FCs of the right frontal regions are mainly decreased by inducing mental stress. In contrast, the inter-hemispheric FCs of the left frontal regions are increased at the delta, theta, alpha and beta bands, and significantly decreased at the lower gamma band. Interestingly, even after 20 minutes of recovery, some reactivity occurred and the tempro-parietal connections, especially at the theta and beta bands, significantly changed after the release of stressors. The release of stressors presented a recovery pattern of inter-hemispheric FCs mainly observed at the homologous inter-hemispheric FCs in fronto-central regions at the lower gamma band, and in tempro-parietal regions at the delta band. In addition, the significant changes in the FCs express correlation with changes in cortisol and VAS.

In fact, the steroid hormone of cortisol almost has receptors in every cell in the body that enable it to activate them depend on the cell type including those in the brain to regulate metabolism and restore the homeostasis (McEwen 2017). The cortisol could pass the blood-brain barrier and reach to its related receptors in the cortex, limbic system, hippocampus, thalamus, and hypothalamus (Dallman 2005). Hyperpolarization of the membrane has been suggested to be the primary mechanism for fast effect of cortisol (15 to 20 min) (Makara and Haller 2001). The hyperpolarization is associated with neuronal silence that could influence the connectivity of a neuronal network (Wrosch, Von Einem et al. 2017). In stressful condition, this effect has been observed that several brain regions including the hippocampus, amygdala, and the prefrontal cortex (PFC) and their interactions are influenced (Yaribeygi, Panahi et al. 2017). As the consequence, cognitive functioning, emotional regulation, and self-regulatory behaviors will be affected (Ursin and Eriksen 2004, McEwen 2007, Erfani, Sahraei et al. 2016, Yaribeygi, Panahi et al. 2017). Moreover, it is also important to return back to an initial state after removing of the stressor to prevent the overload stress and may advancement to chronic stress (McEwen

2017). Since the autonomic nervous system is managed by the hemispheric lateralization (Lee, Gerdes et al. 2014), therefore, bihemispheric association must be critical in the recovery phase which will be discussed in the following.

Previous studies have shown that the salivary cortisol, VAS, heart rate variation features, the linear and non-linear features and networks features of EEG changed significantly following TSST (Ghahvehchi-Hosseini, Manshadi et al. 2018, Mohammadi Alireza, Asgar Emamgoli et al. 2018, Lotfan, Shahyad et al. 2019). The relative alpha band power (8-10 Hz) increased after stress in the eye closed in the all channels in the same pattern of cortisol change(Ghahvehchi-Hosseini, Manshadi et al. 2018). Besides, the theta/beta ratio also decreased (Putman, Verkuil et al. 2013) and asymmetry of functionality in the prefrontal cortex will be raised towards the right hemisphere (Seo and Lee 2010). Moreover, a decrease of alpha band power and an increase of beta band power and their associations with changes in heart rate variability and cortisol level have been reported (Hamid, Sulaiman et al. 2010, Seo and Lee 2010). The imaging study showed the activity of the dorsolateral prefrontal cortex (DLPFC) is decreased under short-term mental stress (Hermans, Henckens et al. 2014). These findings demonstrate the complexity of an underlying mechanism that could influence a widely distributed FCs in the brain network.

In the same line with the previous studies, we also observed that an active induction of mental stress could significantly decrease the right frontal inter-hemispheric FCs and increase the left frontal inter-hemispheric FCs at the delta, theta, alpha and beta bands while decreasing it at the lower gamma band. For the survival matter, automatic responses need to be suppressed after mentally stressful situations. Considering the role of the right side of the brain as the main manager of the sympathetic response (Lee, Gerdes et al. 2014), therefore, interactions of the right hemisphere with other parts of the brain must be decreased. One possible interpretation of this mechanism is that cortisol facilitates the hyperpolarization of the neural cell membranes which is associated with neuronal silence and exerts a top-down inhibition on subcortical regions (Wager, Davidson et al. 2008) such as amygdala. Subsequently, the network of hypothalamus-pituitary and adrenal glands are activated which is managed by the left hemisphere to dampen the stress response. Therefore, the left frontal interactions should also increase in this study paradigm. Furthermore, significant correlations between changes of FCs and changes of VAS and cortisol values suggests that amount of changes in the interhemispheric connectivity may be associated with the induced stress level.

On the other hand, the release of stressors also presented a recovery pattern of inter-hemispheric FCs. The inter-hemispheric FCs of the right frontal region increased at the delta, theta and gamma bands, while FCs of the left frontal regions were decreased at the delta and alpha band. Moreover, the inter-hemispheric FCs of the right parietal regions also increased at the theta band, and the inter-hemispheric FCs of the left parietal region decreased at the beta band which could be related to reformation of attention network during the stress release.

Limitations

We cannot thoroughly exclude the variability of the FC measures based on the task-free EEG data in various stages of the experiment. However, paired comparisons in a controlled environment and implying a restricted threshold (FWE corrected p-values) could reduce the risk of over-interpretation. In addition, only male subjects were recruited in this study that makes it inconceivable to apply the results to female subjects. Gender differences in response to stress are considered a major issue (Kudielka, Buske-Kirschbaum et al. 2004). Furthermore, this study might perform with more sample size to improve the power of calculations. Also, investigation of task-based EEG while subjects are exposed to stress stimuli could provide more information on the dynamic of FC changes as well.

Conclusions

Our findings reveal that exposure to short-term psychological stress changes the cortisol level and brain functional connectivity pattern that has a clear sign on the behavior measured by the visual analogue scale in this study. Based on the results, we think the bihemispheric association plays an important role to adapt and cope with stressful conditions.

Conflict of interest

Authors declare no conflict of interest.

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List of Tables and Figures

Table 1. The most significant changes of functional connectivity in eyes-open condition (p-value <0.05, FWE corrected)

Statistical comparison	Pairs of electrodes	Frequency band	T value	p-value uncorrected
Before versus after stress induction	P3-F7	Delta	-4.77	0.00015
	F3-C3	Beta	4.87	0.00012
After recovery versus after stress induction	Fpz-F3	Beta	4.70	0.00017
	TP8-T5	Theta	5.48	0.000032

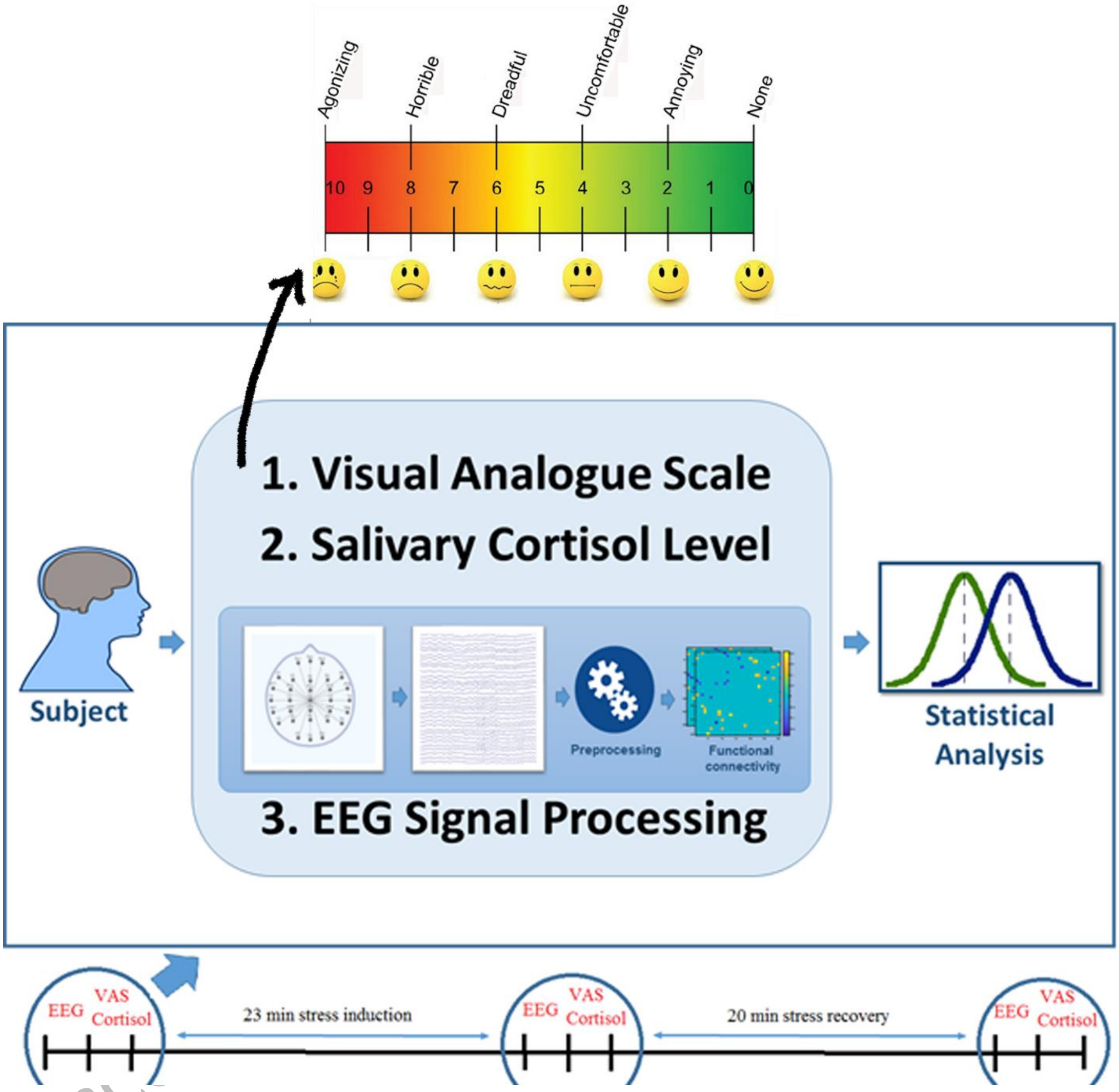

Figure1. Experimental design

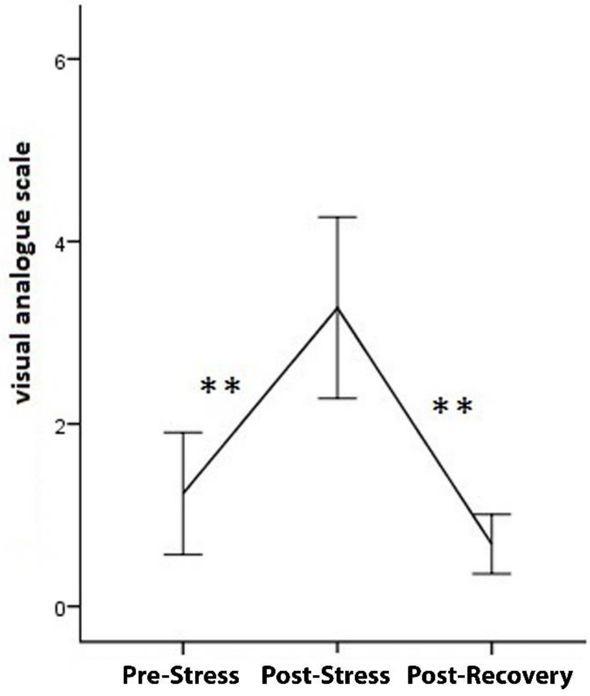

Figure 2. significant different of VAS after stress in comparison to befor stress and after recovery. **: p-value < 0.00001

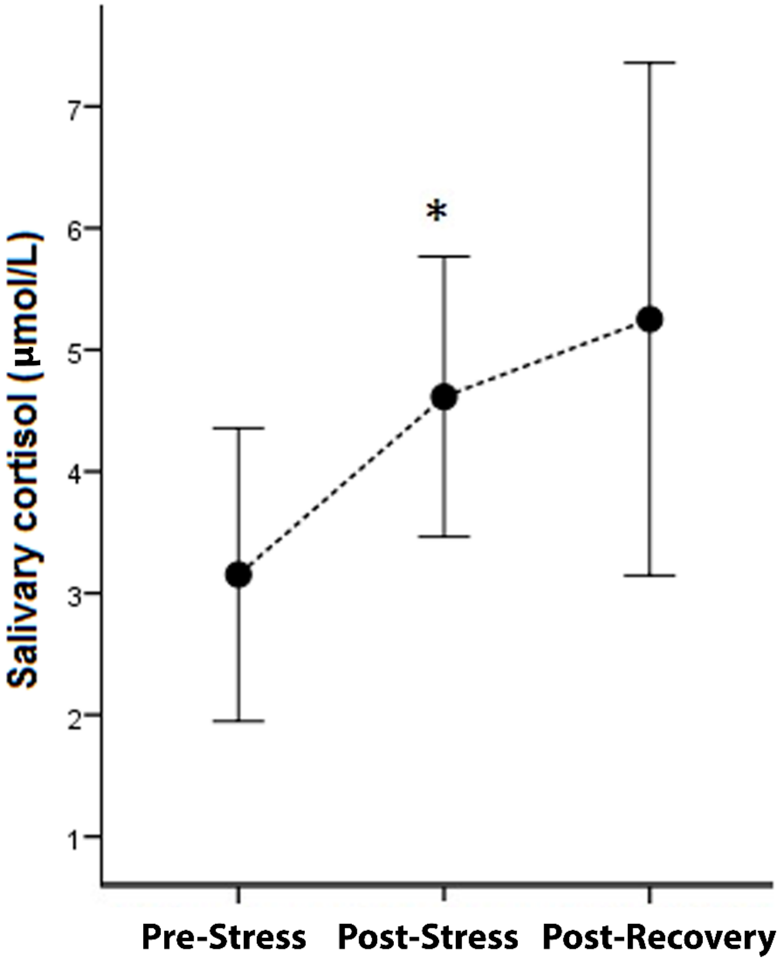

Figure 3. The subjects' salivary cortisol levels was increased after stress to compare before stress. *: *p* value<0.05

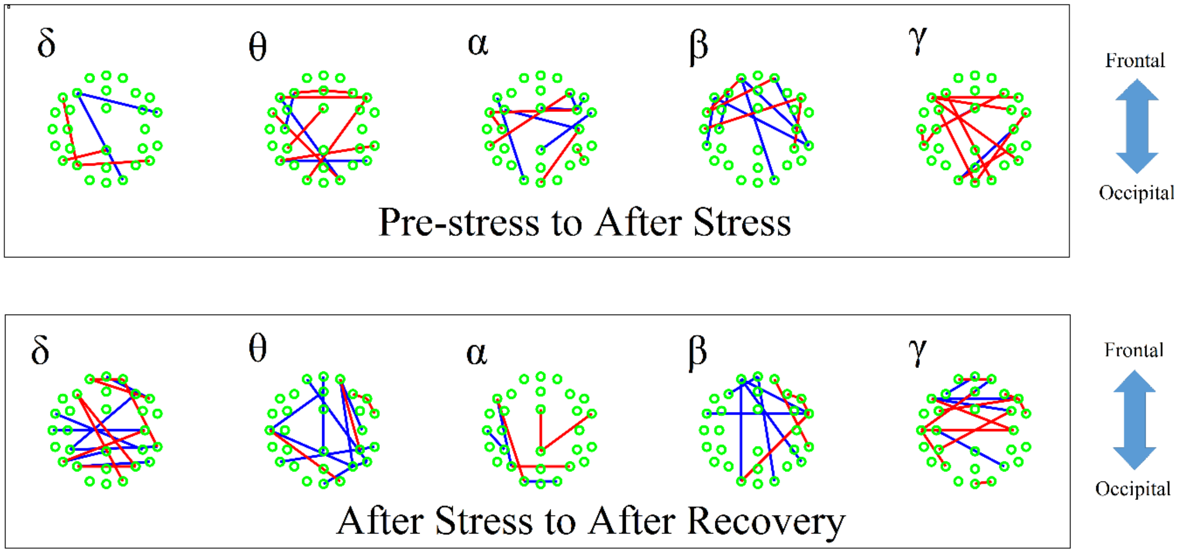

Figure4. Significant changes of functional connectivity in eyes open condition (p-value <0.05, FDR corrected). Blue lines indicate a significant increase and red lines show a significant decrease in FCs. The frequency bands presented are delta [1-4 Hz], theta [4-8 Hz], alpha [8-13 Hz], beta [13-30 Hz], and lower-gamma [30-40 Hz].

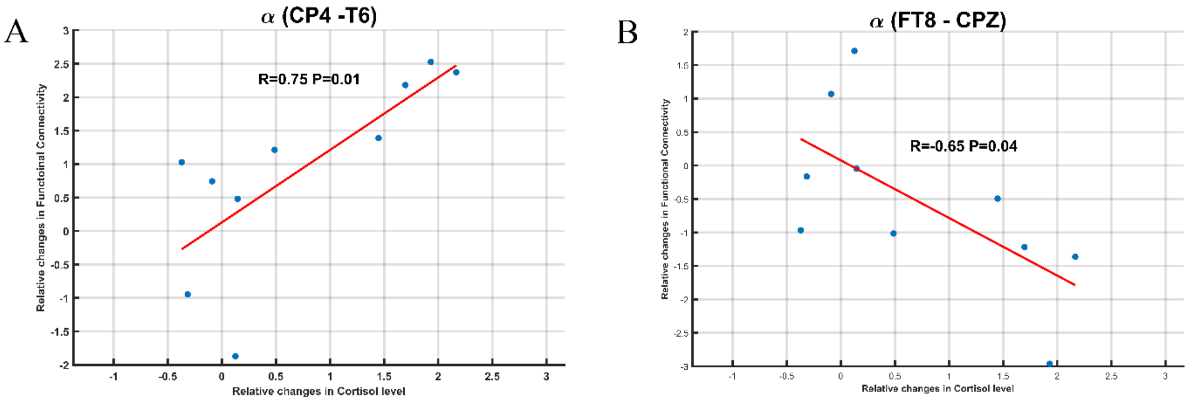

Figure5. Association between relative changes of FCs in eyes open condition and relative changes of cortisol level (A) After inducing to short-term mental stress (B) Recovery phase. It should be mentioned that only subjects with no missing data are presented (10 subjects) in this figure.