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

Authors: Zahra Rezvani¹, Reza Khosrowabadi¹, Afrooz Seyedehabahmi¹, Golam-Hossein Meftahi², Boshra Hatef²*

¹Institute for Cognitive and Brain Sciences, Shahid Beheshti University GC, Tehran, Iran; ²Neuroscience Research Center, Baqiyatallah University of Medical Science, Tehran, Iran.

*Corresponding author: Boshra Hatef

Address: Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Vanak Square, Molla-Sadra Avenue, Tehran 19945, Iran.

Email: boshrahatef@bmsu.ac.ir

To appear in: Basic and Clinical Neuroscience

Received date: 2020/04/26
Revised date: 2020/06/29
Accepted date: 2020/07/6

This is a “Just Accepted” manuscript, which has been examined by the peer-review process and has been accepted for publication. A “Just Accepted” manuscript is published online shortly after its acceptance, which is prior to technical editing and formatting and author proofing. Basic and Clinical Neuroscience provides “Just Accepted” as an optional and free service which allows authors to make their results available to the research community as soon as possible after acceptance. After a manuscript has been technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as a published article. Please note that technical
editing may introduce minor changes to the manuscript text and/or graphics which may affect the content, and all legal disclaimers that apply to the journal pertain.

Please cite this article as:
Rezvani, Z., Khosrowabadi, R., Seyedehrahimi, A., Meftahi, G. H., & Hatef, B. (In Press). Alteration of Brain Functional Network and Cortisol Level During Induction and Release of Stress: An EEG Study in Young Male Adults. *Basic and Clinical Neuroscience*. Just Accepted publication Jul. 13, 2020. Doi:10.32598/bcn.2021.2525.1
DOI:10.32598/bcn.2021.2525.1
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, the release 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 of the 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 \((\text{FC}_{\text{after stress}}-\text{FC}_{\text{before}})/\text{FC}_{\text{before}}\)] and relative changes in Cortisol [for instance \((\text{Cortisol}_{\text{after stress}}-\text{Cortisol}_{\text{before}})/\text{Cortisol}_{\text{before}}\)] and VAS [for instance \((\text{VAS}_{\text{after stress}}-\text{VAS}_{\text{before}})/\text{VAS}_{\text{before}}\)] 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 Figure 4). In addition, 20 minutes of recovery causes a significant change at the temporo-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 temporo-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 temporo-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
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.

**References**

Achard, S., R. Salvador, B. Whitcher, J. Suckling and E. Bullmore (2006). "A resilient, low-frequency, small-world human brain functional network with highly connected association cortical hubs." Journal of Neuroscience 26(1): 63-72.
Alonso, J., S. Romero, M. Ballester, R. Antonijoan and M. Mañanas (2015). "Stress assessment based on EEG univariate features and functional connectivity measures." Physiological measurement 36(7): 1351.
Baba, K., R. Shibata and M. Sibuya (2004). "Partial correlation and conditional correlation as measures of conditional independence." Australian & New Zealand Journal of Statistics 46(4): 657-664.
Baum, A. and R. Contrada (2010). The handbook of stress science: Biology, psychology, and health, Springer Publishing Company.
Benjamini, Y. and Y. Hochberg (1995). "Controlling the false discovery rate: a practical and powerful approach to multiple testing." Journal of the royal statistical society. Series B (Methodological): 289-300.
Dallman, M. F. (2005). "Fast glucocorticoid actions on brain: back to the future." Frontiers in neuroendocrinology 26(3-4): 103-108.
Dickerson, S. S. and M. E. Kemeny (2004). "Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research." Psychological bulletin 130(3): 355.
Erfani, M., H. Sahraei, Z. Bahari, G. H. Meftahi, B. Hafez, A. Mohammadi and S. H. Hosseini (2016). "Evaluation of the effect of time change in cognitive function in volunteers in Tehran." Global Journal of Health Science 9(2): 119.
Friston, K. J. (1994). "Functional and effective connectivity in neuroimaging: a synthesis." Human brain mapping 2(1-2): 56-78.
Garmezy, N. E. and M. E. Rutter (1983). Stress, coping, and development in children. Seminar on Stress and Coping in Children, 1979, Ctr for Advanced Study in the Behavioral Sciences, Stanford, CA, US, Johns Hopkins University Press.

Ghahvehchi-Hosseini, F., E. Manshadi, A. Mohammadi, J. Pirzad-Jahromi and B. Hatef (2018). "Evaluation of the Persistence Effect Acute Social Stress Test on the Alpha Band Power." Journal Mil Med 20(5): 509-518.

Hamid, N. H. A., N. Sulaiman, S. A. M. Aris, Z. H. Murat and M. N. Taib (2010). Evaluation of human stress using EEG Power Spectrum. Signal Processing and Its Applications (CSPA), 2010 6th International Colloquium on.

Hellhammer, J. and M. Schubert (2012). "The physiological response to Trier Social Stress Test relates to subjective measures of stress during but not before or after the test." Psychoneuroendocrinology 37(1): 119-124.

Hermans, E. J., M. J. A. G. Henckens, M. Joëls and G. Fernández (2014). "Dynamic adaptation of large-scale brain networks in response to acute stressors." Trends in Neurosciences 37(6): 304-314.

Hüther, G. (1998). "Stress and the adaptive self-organization of neuronal connectivity during early childhood." International Journal of Developmental Neuroscience 16(3-4): 297-306.

Khosrowabadi, R. (2018). "Stress and Perception of Emotional Stimuli: Long-term Stress Rewiring the Brain." Basic and Clinical Neuroscience 9(2): 107-120.

Khosrowabadi, R., C. Quek, K. K. Ang, S. W. Tung and M. Heijnen (2011). A Brain-Computer Interface for classifying EEG correlates of chronic mental stress. IJCNN.

Kirschbaum, C., K.-M. Pirke and D. H. Hellhammer (1993). "The 'Trier Social Stress Test'—a tool for investigating psychobiological stress responses in a laboratory setting." Neuropsychobiology 28(1-2): 76-81.

Kudielka, B., A. Buske-Kirschbaum, D. Hellhammer and C. Kirschbaum (2004). "HPA axis responses to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: impact of age and gender." Psychoneuroendocrinology 29(1): 83-98.

Lang, E. W., A. M. Tomé, I. R. Keck, J. Górriz-Sáez and C. G. Puntonet (2012). "Brain connectivity analysis: a short survey." Computational intelligence and neuroscience 2012: 8.

Lee, S. W., L. Gerdes, C. L. Tegeler, H. A. Shaltout and C. H. Tegeler (2014). "A bihemispheric autonomic model for traumatic stress effects on health and behavior." Frontiers in psychology 5: 843.

Lewis, R. S., N. Y. Weekes and T. H. Wang (2007). "The effect of a naturalistic stressor on frontal EEG asymmetry, stress, and health." Biological psychology 75(3): 239-247.

Lotfan, S., S. Shahyad, R. Khosrowabadi, A. Mohammadi and B. Hatef (2019). "Support vector machine classification of brain states exposed to social stress test using EEG-based brain network measures." Biocybernetics and Biomedical Engineering 39(1): 199-213.

Lupien, S. J., B. S. McEwen, M. R. Gunnar and C. Heim (2009). "Effects of stress throughout the lifespan on the brain, behaviour and cognition." Nature reviews. Neuroscience 10(6): 434.

Makara, G. B. and J. Haller (2001). "Non-genomic effects of glucocorticoids in the neural system: evidence, mechanisms and implications." Progress in neurobiology 65(4): 367-390.

McEwen, B. S. (2007). "Physiology and neurobiology of stress and adaptation: central role of the brain." Physiological reviews 87(3): 873-904.

McEwen, B. S. (2017). "Neurobiological and systemic effects of chronic stress." Chronic Stress 1: 2470547017692328.

McEwen, B. S., N. P. Bowles, J. D. Gray, M. N. Hill, R. G. Hunter and I. N. Karatsoreos (2015). "Mechanisms of stress in the brain." 18(10): 1353-1363.

Mohammadi Alireza, Asgar Emamgoli, Maryam Shirinkalam, GolamHossein Meftahi, Shima Shahyad, Keyvan Yagoobi and Boshra Hatef (2018). "the persistent response to acute psychosocial stress." Biomedical Signal Processing and Control.
Peters, A., B. S. McEwen and K. Friston (2017). "Uncertainty and stress: Why it causes diseases and how it is mastered by the brain." Prog Neurobiol 156: 164-188.

Putman, P., B. Verkuil, Arias-Garcia E, Pantazi I and v. S. C (2013). "EEG theta/beta ratio as a potential biomarker for attentional control and resilience against deleterious effects of stress on attention." Cogn Affect Behav Neurosci.

Putman, P., B. Verkuil, E. Arias-Garcia, I. Pantazi and C. van Schie (2014). "EEG theta/beta ratio as a potential biomarker for attentional control and resilience against deleterious effects of stress on attention." Cognitive, Affective, & Behavioral Neuroscience 14(2): 782-791.

Schoffelen, J. M. and J. Gross (2009). "Source connectivity analysis with MEG and EEG." Human brain mapping 30(6): 1857-1865.

Seo, S.-H. and J.-T. Lee (2010). Stress and EEG. Convergence and hybrid information technologies, InTech.

Shaffer, J. P. (1995). "Multiple hypothesis testing." Annual review of psychology 46(1): 561-584.

Tegeler, C. H., H. A. Shaltout, C. L. Tegeler, L. Gerdes and S. W. Lee (2015). "Rightward dominance in temporal high-frequency electrical asymmetry corresponds to higher resting heart rate and lower baroreflex sensitivity in a heterogeneous population." Brain and behavior 5(6): e00343.

Ursin, H. and H. R. Eriksen (2004). "The cognitive activation theory of stress." Psychoneuroendocrinology 29(5): 567-592.

Wager, T. D., M. L. Davidson, B. L. Hughes, M. A. Lindquist and K. N. Ochsner (2008). "Prefrontal-subcortical pathways mediating successful emotion regulation." Neuron 59(6): 1037-1050.

Wang, Y., J. Kang, P. B. Kemmer and Y. Guo (2016). "An efficient and reliable statistical method for estimating functional connectivity in large scale brain networks using partial correlation." Frontiers in neuroscience 10: 123.

Wrosch, J. K., V. Von Einem, K. Breininger, M. Dahlmanns, A. Maier, J. Kornhuber and T. W. Groemer (2017). "Rewiring of neuronal networks during synaptic silencing." Scientific reports 7(1): 11724.

Yaribeygi, H., Y. Panahi, H. Sahraei, T. P. Johnston and A. Sahebkar (2017). "The impact of stress on body function: A review." EXCLI JOURNAL 16: 1057-1072.

Zigmond, M. J. and F. E. Bloom (1999). "Fundamental neuroscience."

**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            |
Figure 1. Experimental design
Figure 2. Significant different of VAS after stress in comparison to before 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.
**Figure 4.** 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].

**Figure 5.** 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.