Short Communication

The Complexity of Electroencephalographic Signal Decreases during the Social Stress

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Abstract

Social stress affects brain function. Trier social stress test (TSST) is a standard test to assess it. The study aimed to analyze the electroencephalographic (EEG) recording during and after TSST in healthy subjects. The EEG signals of 44 healthy men participating in the study were recorded in the control condition, during and after TSST and after 30 min of recovery. Salivary cortisol (SC) and the Emotional Visual Analog Scale (EVAS) score were measured in the control condition, after TSST, and after the recovery period. The false discovery rate correction was used to control the false positive of significance in EEG. In the comparison control condition, the SC and EVAS levels significantly increased after TSST. The relative Delta band frequency significantly increased during TSST. On the other hand, the Beta bands and, in less amount, the Theta and Gamma 1 (30–40 Hz) oscillations decreased, especially in the frontal region. The nonlinear features such as, approximate and spectral entropy, Katz fractal dimension behaved like Beta band oscillation. All changes returned to baseline after TSST except the increase of Katz in the F3 channel after the recovery period. Thus, stress on EEG increased low frequency (1–4 Hz), decreased high frequency (13–40 Hz), and complexity indices during TSST.

Keywords: *Complexity, electroencephalographic, salivary cortisol, trier social stress test*

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Introduction

Human beings are often under stress. Stress has a wide range of effects on the memory and cognitive function of the brain and nearly most of the other organs of the body.[1] These effects follow a u-inverted shape relationship between the amount of stress and performance. Then stress has a beneficial effect if a few or low repetitions and a harmful if much or high repetitions.[2,3] One of the most common psychosocial stress is presenting some things, answering questions in front of others, and receiving negative feedback. This stressor has been modeled in the trier social stress test (TSST).[4] In this situation, the person feels anxiety and becomes ashamed.

On the other hand, he/she should attend and use high cognitive performance to do his/her task or duty in the perfect form. If this stress is much more as distress, the cognitive performance decreases.[2] Previous studies have shown that TSST significantly increases cortisol, continuing to rise even after 20 min of recovery.[5] The heart rate increases during the stress test, returning to normal after TSST.[6] However, some nonlinear features of the heart rate variation remain decreased even after 20 min of recovery, which indicates that stress has long-term effects on the cardiovascular and hormonal systems.[5] There have been very few studies were done about the effect of TSST on brain activity[7] which found that immediately and also 20 min after the TSST test, concurrently with the elevation of cortisol levels, the relative power of the Alpha 1 band frequency in the closed eye was markedly increased in all parts of the regions and remained elevated even after the recovery periods. The Katz fractal dimension of the electroencephalographic (EEG) signals in the closes eye significantly decreased after the TSST in the prefrontal region and returned to baseline after the recovery period. The positron-emission tomography study showed that a high glucose metabolic rate in the lateral aspects of the superior

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frontal gyrus, amygdala, and hippocampus is associated with increased cortisol levels after TSST. The increase of cortisol was also accompanied by the decrease of rostral of the medial prefrontal cortex (PFC) activity.[8] The functional near-infrared spectroscopy (FNIRS) study during the TSST showed increased activity in the dorsolateral prefrontal cortex (DLPFC), inferior frontal gyrus (IFC), and somatosensory association cortex, especially on the left side compared to control conditions in which the person is either reading numbers or solving simple mathematics.[9] Several studies have shown that the right hemisphere, especially the right PFC, is activated in negative emotions and is correlated with the cortisol level.[10-12] The hormonal and brain activity change findings due to the Montreal Imaging Stress Task (MIST) were not similar to the TSST. A person calculates the math in 10 min in the MIST while receiving a negative and stressful feedback message from the computer, not another person.

Social stress was not applicable in this test. The study showed that the salivary alpha-amylase and heart rate had raised during the MIST.[13,14] The combination assessment of the EEG and FNIRS during MIST showed an increase in the power of Beta and a decrease in the Alpha Band frequency of the PFC region. The FNIRS showed that the cortical activation of the right PFC also decreased.[13] Another similar study showed that the power of the frequency of the Gamma band increased in the PFC during MIST. It had a significant direct relationship with increased heart rate and expected stress levels.[14] The combined EEG-FNIRS recording during negative emotion demonstrated that an increase of brain activity based on the FNIRS was seen in the right frontal region. An increase of relative power of the Delta band frequency based on EEG was also seen.[15] A study that examines the change in brain activity based on the EEG has not been conducted yet. The current study aimed to record the EEG during and after TSST and assess the signal complexity in addition to spectral analysis. One of the present study innovations was related to the measurement of such regions, reported in a previous artificial intelligence study proven essential to interact with cortisol.[11] The hypothesis was that the EEG signals during and after the stress trend to low frequency and complexity, and the TSST, unlike MIST, do not increase the Beta and Gamma band of EEG because the negative mood overcomes mental activity conditions pressure on a person.

Materials and Methods

Participants

This study was performed on 44 healthy male volunteers. The inclusion criteria included no systemic diseases such as hypertension and diabetes, no mental disorders under the supervision of a physician and receiving psychiatric drugs, no use of the narcotic drug, absence of severe stressors (death of a family member, job loss) in the past 3 months, and not being an athlete. Individuals signed a written consent to participate in the study. The study had an ethical code of IR. BMSU. REC.1398.141.

Procedure

Participants were asked to have a good night sleep the night before the test and not have severe stress. Everyone is tested between 10 and 15 o'clock because cortisol levels slightly fluctuate in this time frame. Participants were asked not to eat anything for 1 h before the test. The 0.5 cc of saliva sample was taken to measure salivary cortisol (SC) levels, and the level of stress that the person reported was asked based on Emotional Visual Analog Scale (EVAS)[16] before and after the TSST and after 30 min of recovery. The EEG electrodes based on a standard procedure were connected to a cap on the head. The EEG was recorded before, during the TSST (ten minutes after TSST), and after 30 min of recovery. The schematic steps of the study are presented in Figure 1. The baseline EEG recording and saliva sampling were considered to control conditions. Since the whole time of the test was around 1 h, the change of cortisol and brain state was not expected. The TSST had three parts. First, the subjects waited for 5 min. After that, they went to the test room and stood in front of two men examiners with serious appearances and introduced themselves for 2 min. Finally, they were asked to subtract the number 13 from 1022 repeatedly to reach zero for 8 min. If they answered incorrectly, they had to start calculation from 1022.[16] The Depression Anxiety Stress Scale (DASS)-42 item questioner[17] was taken after recording to determine participants' emotional state.

Salivary cortisol measurement

The saliva samples were taken minimally (0.5 mL). They were kept in a −80°C refrigerator. The human saliva cortisol enzyme immunoassay kit from the IBL Company made in Germany was used for the procedure based on its instructions. The negative curve between cortisol and optical density adjusted on the kit curve was used in each sample. The range of cortisol in this kit was 0.015–3 μg/dL. The level of cortisol of saliva was reported in nmol/L. The standard normal range mentioned in this kit brochure is 0.9–9.2 nmol/L at 9–15 o'clock.

Electroencephalographic recording and analysis

The EEG instrument used in this study is from the Liv Intelligent Technology Company made in Iran. The characteristics of this instrument include input impedance: 10 M, bandwidth: 2 kHz, gain: 1, and resolution: 24bit. The EEG cap and silver–silver chloride electrodes were used. The electrodes of F3, F4, F7, F8, T8, P4, and O2 were recorded, and the reference electrode was Cz. The impedance of the electrodes diminished under 15 kΩ by using the ECG gel and cleaning the skin with alcohol. The person was asked to be calm and not move during the recording. One minute eye open and 1 min eye closed were recorded in each

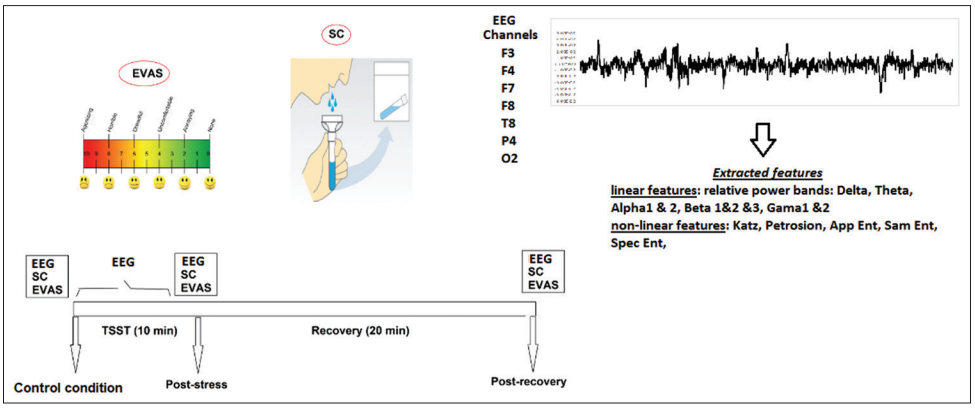

Figure 1: The steps of study and given variables

step (before, after TSST, and after recovery) and during the TSST. The sampling frequency was 256 samples per second. The MATLAB 2014 was used for analysis.

Preprocessing included signal filtering between the 0.2–48 Hz band, visual inspection of individual signals and removal of motion artifacts, electromyogram and blinking by the ICA method, and linear analysis of relative power extraction (power of each band to the sum of bands) by Welch method with hanging window 10 s and 50% overlap and frequency resolution of 0.25 Hz. These bands included Delta: 0–4 Hz, Theta: 4–8 Hz, Alpha 1: 8–10 Hz, Alpha 2: 10–12 Hz, Beta 1: 12–16 Hz, Beta 2: 16–20 Hz, Beta 3: 20–30 Hz, Gamma 1: 30–35 Hz, and Gamma 2: 35–40.[18,19] The studied nonlinear indices included sample entropy (SampEn) and approximate entropy (AppEn) in the time domain,[18,20] spectral entropy (SpEn) in the frequency domain,[21] and the fractal dimension of Petrosian and Katz.[22] All indices were calculated for both open and closed eyes separately.

Statistics

First, the variables were examined for normal distribution by the Kolmogorov–Smirnov test. The ANOVA repeated measurements. Furthermore, for pairwise comparison, the Bonferroni correction was used to compare the EVAS score, cortisol levels, and linear and nonlinear EEG indices (which had a normal distribution) between the three steps: control condition, after TSST, and after recovery. Since EEG channels are related together, the accepted amount of the EEG data was considered after calculating the false discovery rate correction (FDR) below 0.05. Then, F and *P* values reported by statistical analysis did not know enough to report significantly.

Results

The properties of the participants in the study are given in Table 1. They were health subjects mentally based on the DASS score, and none of them had a score more than health level. Impairment of mental state based on the DASS score starts from 15 for stress, 8 for anxiety, and 10 for depression.[23]

Table 1: The properties of participants

Variable	Amount
n	44
Age	30.45 (8.2)
Weight	80.25 (16.2)
Height	176.86 (7.3)
Have a job (%)	68.2
Cortisol baseline (ng/dl)	7.94 (3.5)
DASS (stress/anxiety/depression)	10.9 (7)/7.3 (5)/8.5 (6)

DASS - Depression Anxiety Stress Scales

Figure 2 shows that the cortisol level and the EVAS score significantly increased after the TSST compared to the control condition. The EVAS score returned to baseline after recovery, but the cortisol level was still upward after recovery.

Electroencephalographic changes during trier social stress test

Comparing the changes in the relative amount of EEG bands after calculating the FDR correction between control condition during TSST and poststress and postrecovery in open eye showed a significant increase in the relative power of the Delta band frequency during the TSST. However, it returns to its baseline after stress in all the channels [Figure 3]. The Beta band frequencies significantly reduced during the TSST compared to before and after stress, especially in the frontal region [Figure 4]. The Theta and, in a lower amount, the Gamma 1 band frequencies also decreased during the TSST but not in all channels [Figure 5]. The Gamma 2 band did not show any significant changes. The Alpha bands seem to have a transitory behavior in response to stress. The relative power of the Alpha 1 band increased significantly only in the F4 channel during step 1 of the TSST compared to the control condition and poststress (FDR $P < 0.05$). The relative power of the Alpha 2 band showed an inverse pattern and decreased during the TSST but not significantly (expect in step one of the TSST to compare control condition [FDR $P < 0.05$]).

The nonlinear indices comparisons showed that KATZ, spectral entropy, sample entropy, and approximate entropy indices decreased significantly during TSST compared to control conditions and poststress and postrecovery in most channels. The changes in the O2 and P4 channels were less and sometimes did not reach a significant level [Figure 5]. The Petrosian index showed minor change and reversed behavior in the front and behind regions. Hence, the results showed that stress decreased the Petrosian index significantly in the F3 and F4 channels and increased it in T8 and O2 to compare the control condition (FDR $P < 0.05$).

Electroencephalographic changes after trier social stress test

No significant change was seen between poststress and control conditions in the relative power of all of EEG band frequencies in the open and closed eye recording. The changes of nonlinear features were not significant except for the increase of fractal dimension of Katz in the F3 channel in the closed eye (FDR $P < 0.05$).

Discussion

This study aimed to evaluate the changes in the brain activity during and after TSST and after 30 min of recovery in terms of linear and nonlinear parameters in those channels that Hatef *et al*. had shown to be sensitive to cortisol concentrations.[11] This study on healthy young men showed that the TSST, as the stress test, significantly increased cortisol levels in individuals and even after recovery to compare control conditions. However, EEG findings showed that significant changes in the spectral analysis of the brain activity were only seen during the TSST. The relative power of the Delta band increased, and the relative power of Theta, Beta, and in less amount, the Gamma bands decreased during TSST. The nonlinear features such as sample, approximate and spectral entropy, and fractal dimension of Katz significantly decreased in most of the channels, especially the frontal channels, according to the relative power of the Beta band. However, the relative power of the Alpha band was in a state of transition and had not significantly decreased or increased except for one or two channels. The nonlinear feature of Petrosian behaved the same as the Alpha band but in an inverted pattern. Only one study conducted by Rosenbaum *et al*. in 2018 showed the increase in oxygenation level, based on FNIRS recording in the two networks of the brain (which are parts of the cognitive control network and dorsal attention network). The brain regions involved in this network are the dorsolateral prefrontal cortex, the IFC, and the superior parietal cortex. The IFC is associated with the regulation of negative affect, as the inhibition of emotional memory and behavior and is correlated with stress rating.[9] Since a high temporal and spatial correlation exists between neuronal activity and blood flow, more blood flow is correlated with more brain activation.[24] Until now, no study has been performed to assess the EEG changes during TSST. Balconi *et al*. in 2015 showed that by assessing brain activity change during negative emotional patterns by combined FNIRS-EEG, an increase of O_2Hb was seen within the right hemisphere (mostly in the right-PFC). The EEG activity, mainly the Delta band oscillation, was also intrinsically associated with the cortical hemodynamic responsiveness to the negative emotional.[15] Some studies have shown that performing the mental activity in which attention is focused on the

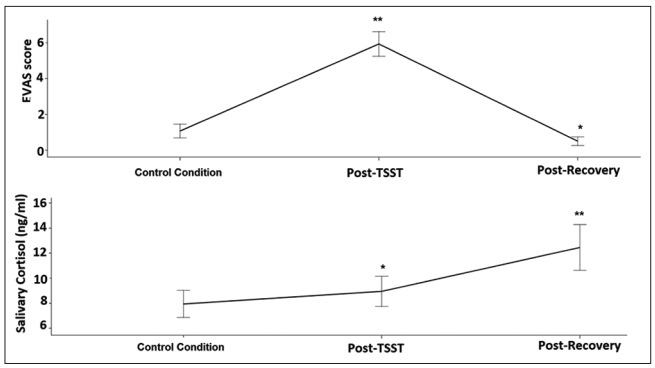

Figure 2: The significant increase of cortisol level and Emotional Visual Analog Scale score after trier social stress test to compare control condition. The Emotional Visual Analog Scale score returned to baseline after recovery, but the cortisol level increased more. *: $P < 0.05$, **: $P < 0.001$. Plot shows mean and 95% confidence interval

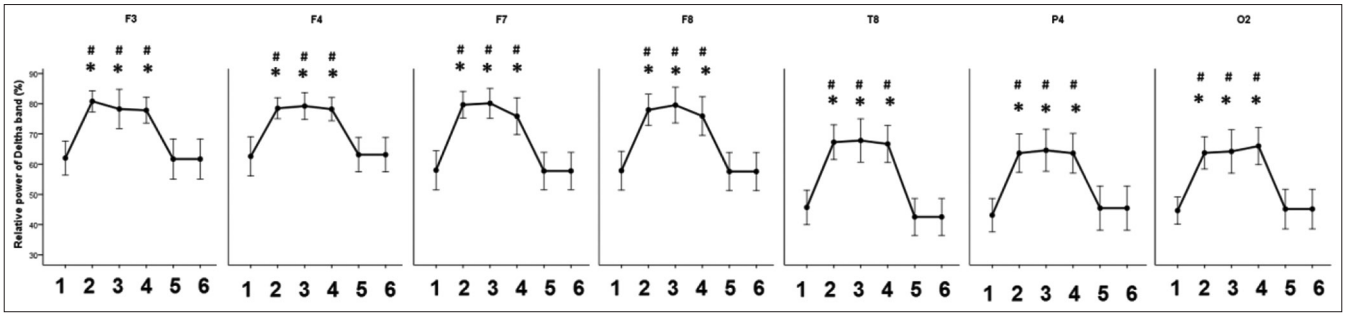

Figure 3: The plot shows a significant increase of relative power of Delta band frequency during trier social stress test in comparison with control condition and poststress and postrecovery in channels of F3, F4, F7, F8, T8, P4, and O2. *: Significant false discovery rate correction $P < 0.001$ from the pairwise comparison between trier social stress test steps and control condition. #: Significant false discovery rate correction $P < 0.001$ from the pairwise comparison between trier social stress test steps and poststress and postrecovery. 1: Control condition, 2: First part of trier social stress test, 3: Second part of T SST, 4: Third part of trier social stress test, 5: Poststress, 6: Postrecovery. Data is mean and 95% confidence intereval

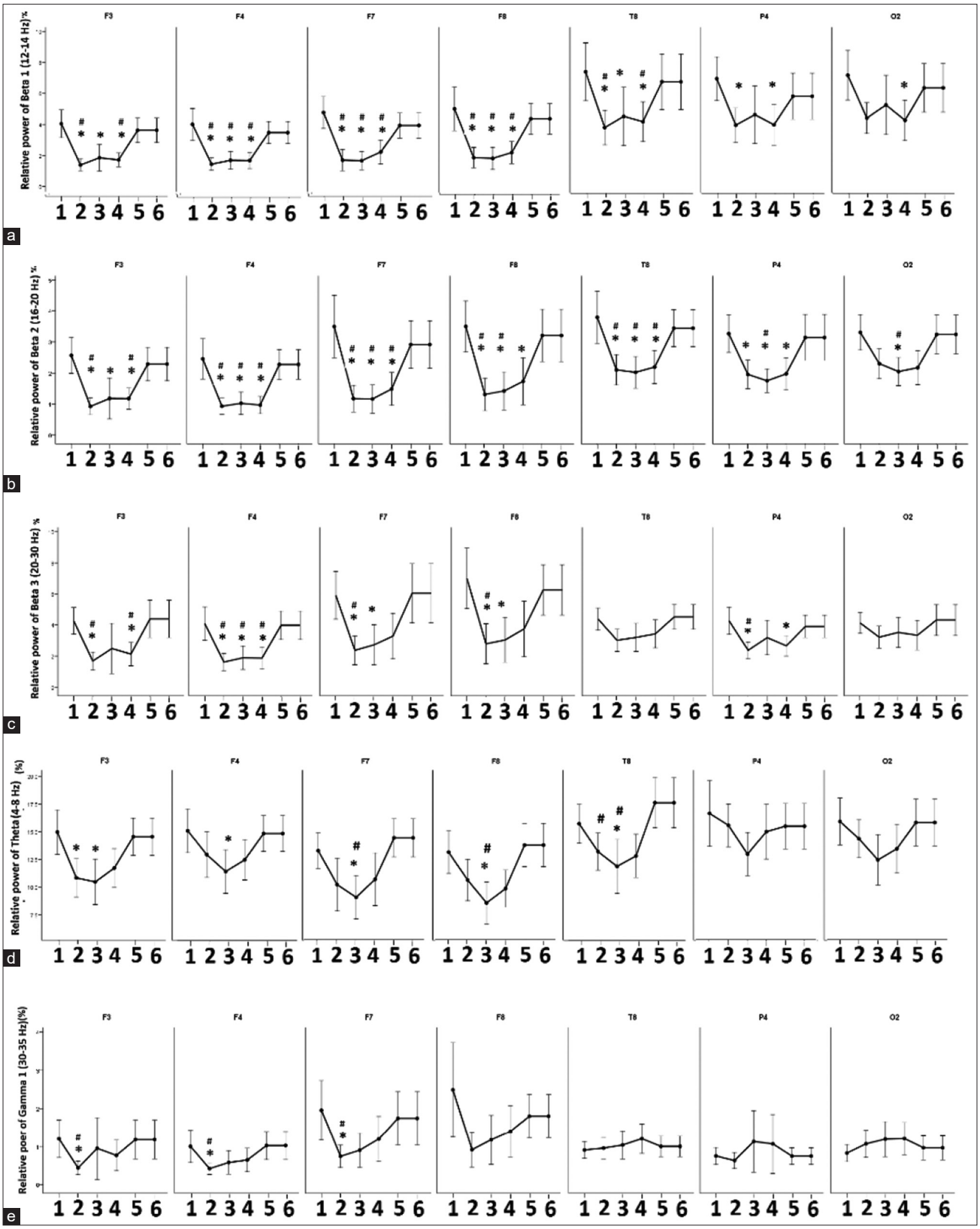

Figure 4: The plot shows a decrease of the relative power of Beta 1 (a), Beta 2 (b), Beta 3 (c) Theta (d), and Gamma 1 (e) band frequency during trier social stress test in comparison with control condition and poststress and postrecovery in channels of F3, F4, F7, F8, T8, P4, and O2. *: Significant false discovery rate correction $P < 0.01$ from the pairwise comparison between trier social stress test steps and control condition. #: Significant false discovery rate correction $P < 0.01$ from the pairwise comparison between trier social stress test steps and poststress and postrecovery. Data is mean and 95% confidence interval. 1: Control condition, 2: First part of trier social stress test, 3: Second part of T SST, 4: Third part of trier social stress test, 5: Poststress, 6: postrecovery. Plot shows mean and 95% confidence interval

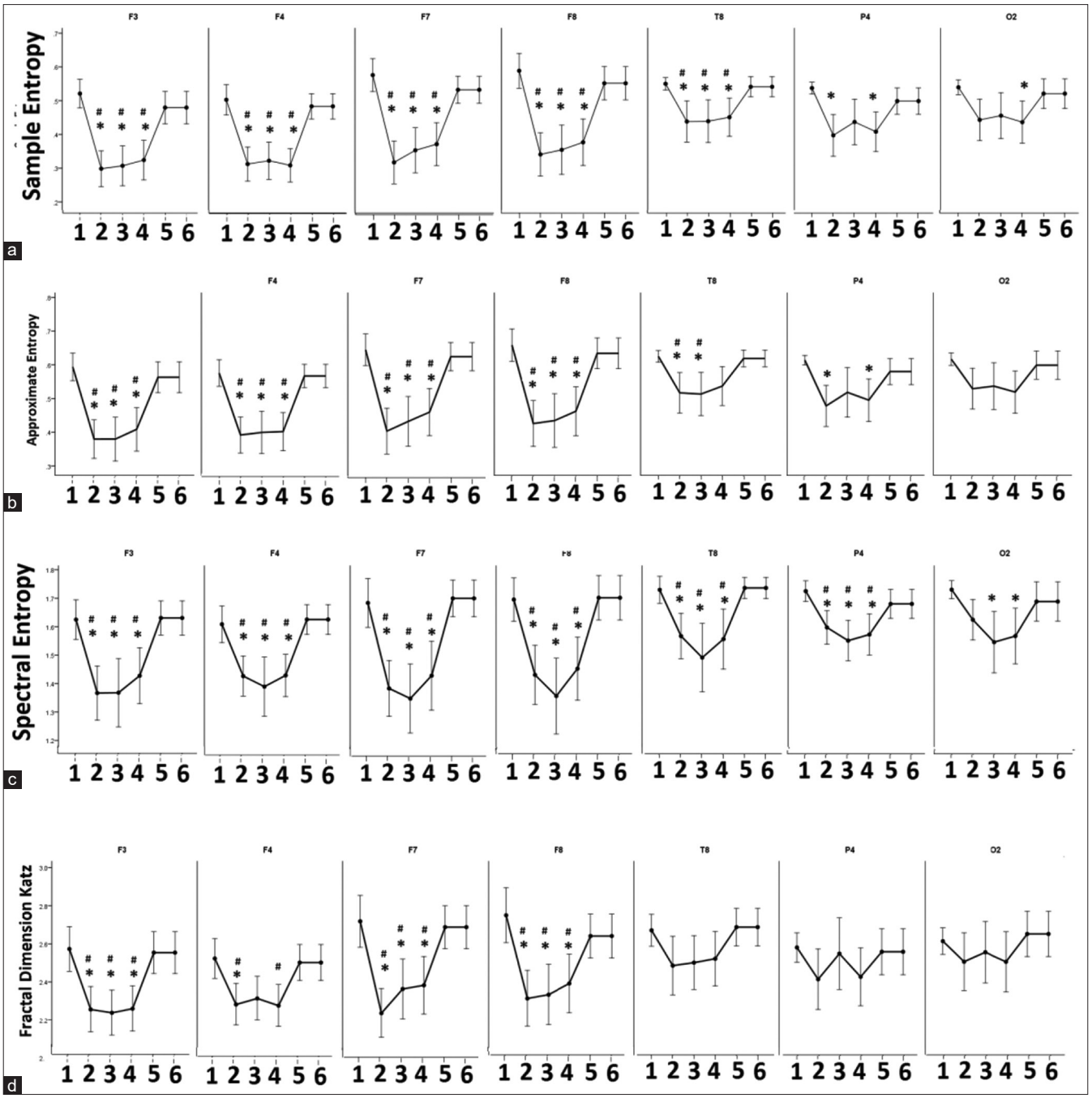

Figure 5: The plot shows a decrease of sample entropy (a), approximate entropy (b) spectral entropy (c), and fractal dimension of Katz (d) during trier social stress test in comparison with control condition and poststress and postrecovery in channels of F3, F4, F7, F8, T8, P4, and O2. *: Significant false discovery rate correction *P* < 0.001 from the pairwise comparison between trier social stress test steps and control condition. #: Significant false discovery rate correction *P* < 0.001 from the pairwise comparison between trier social stress test steps and poststress and postrecovery. Data is mean and 95% CI. 1: Control condition, 2: First part of trier social stress test, 3: Second part of T SST, 4: Third part of trier social stress test, 5: Poststress, 6: Postrecovery. Plot shows mean and 95% confidence interval

inside increases the Delta band frequency.[25,26] Thus, it can be concluded that the TSST test, which is a mental activity with a negative feeling, an increase in the power of the Delta band frequency concurrently with an increase in the O_2Hb might have occurred. Our findings proved this assumption. Based on a previous study,[25] the increase of Delta band frequency in the current study might be related to an internal focus of the participant to find the correct answer and avoid shame in front of examiners. The TSST was used to induce stress in this study and differed from other studies that used the MIST test. It has been observed that salivary alpha-amylase levels and heart rate were elevated, the power of Alpha band decreased and the power of the Beta band increased in the channels located on the forehead, and the oxygen delivery in the right area of the forehead area reduced in the combined EEG-FNIRS

recording during the MIST stress test.[13,27] In another study, the Gamma band oscillation of the brain increased in the PFC region, and this increase was significantly related to an increase in heart rate and expected stress levels. The relationship between relative Gamma power in this region and stress levels was stronger than the Alpha band asymmetry.[14] The reason for this difference may be due to the procedure of these two stress tests. In the MIST test, the person is not confronted with people and performs a series of arithmetic actions on the computer with a time limit. However, in the TSST test, the person is confronted with several examiners and is socially in a state of stress and embarrassment. Therefore, the feeling is sensed, and the activity of the brain is different in these two tests. In addition, in this study, the PFC was not assessed by EEG, and no precise conclusion can be drawn, although the PFC is not expected to behave differently compared to the frontal region. Another challenging point to consider is that the person was seated in the control condition and poststress tests while standing during the TSST. Previous studies examining the effect of different positions on brain activity have shown that standing increased the high frequencies and decreased the low frequencies of EEG,[28-30] while the results of this study were quite different. Then, the effect of stress overcomes the effect of position on brain activity, and it cannot be stated that a portion of the changes seen during the TSST was due to the effect of position on brain activity. The channel of the frontal region was more sensitive to changes during the TSST than the parietal and occipital regions. Although the channels in the nonfrontal areas of the right hemisphere are sensitive to stress,[11,27] it can be concluded that the frontal and prefrontal regions are more involved in stress than others.[9,14,15]

It has been found that nonlinear indices show changes more clearly and distinctly, which again confirms that nonlinear indices more accurately represent the complex behaviors of biological signals.[31] This study showed that the changes of the nonlinear indices were similar to the changes of the relative power of the Beta band frequency[22] that indicated signal complexity was significantly reduced, especially in the frontal channels. Previous studies have shown that changes in the Theta, Beta, and Gamma band frequencies are positively correlated to changes in nonlinear indices that indicate signal complexity.[32,33] It has also been observed that the Katz fractal dimension has significantly increased in the F3 channel in the closed eye after the stress. In a previous study, Hatef *et al*. found that the Katz index value decreased only in the FP channels in the forehead area after the TSST test, which returned to baseline during the recovery phase. In the same study, the relative power of Alpha 1 increased after TSST and even after recovery in the closed eye.[7] However, no significant changes were observed in the linear spectral indices in this study, while the forehead was not examined. A change was seen in the Katz fractal dimension area around the left DLPFC after stress and recovery in the closed eye. This might be due to compensating for the decrease in this index during stress.

Further studies are needed to fully determine how long the effects of stress persist on the electrical brain activity after stress. The classification methods did not use in this study to separate three conditions because for any classification, especially in the EEG based on deep learning, we need very large numbers for train and test,[34] and this study had only 44 cases. The limitations of this study were the low number of channels and the lack of recording from the prefrontal region. This study only evaluates the EEG changes related to stress in healthy men with a mean age of 30.45 (8.2). Then, further studies are needed to assess EEG related to stress conditions in other communities such as women, patients, and elderly or children.

Conclusion

This study showed that the TSST stress, which increased SC, also significantly increased the relative strength of the delta band and decreased the relative strength of Theta, Beta, and Gamma bands in most areas of the head, especially in the frontal region. Furthermore, the nonlinear signals of the signal complexity such as temporal and frequency entropy and fractal Katz had significantly reduced during stress. All of these changes returned to control condition levels after the stress test. Only the Katz index in the F3 channel had increased in the closed eye after stress. This increase remained even after recovery.

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Conflict of interest

There are no conflicts of interest.

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