Original Article

The effects of acute, sub-chronic and chronic psychical stress on the brain electrical activity in male rats

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Abstract

Introduction: Stress is a main factor influencing brain functions as revealed by the electroencephalogram (EEG) recordings. Moreover, different stress durations seemingly cause perturbations in brain waves and lead to mental disorders. This study investigates the effects of acute, sub-chronic and chronic stress on EEG in rats.

Methods: Twenty-eight Wistar adult male rats were randomly allocated to one control and three experimental groups subjected to 6 hr/day of acute (1d), sub-chronic (7d) and chronic (21d) stress. At the end of each period, 20 minutes of EEG recording was taken of each subject.

Results: Percentages of delta, theta and alpha frequencies of the baseline in the chronic stress group showed significant differences from those of the control (P<0.05). Theta waves increased in the chronic stress group compared to the acute and sub-chronic (P<0.05 and P<0.01; respectively) ones. This is while, compared to the control, the acute and sub-chronic stress groups exhibited significantly increased percentages of beta waves (P<0.05 in both).

Conclusion: The data indicate that different stress durations have different impacts on the EEG rhythm. Acute and sub-chronic stress durations led to changed cortical activity, indicating the inability of the subjects to cope with the stress imposed. Also, chronic stress caused irregularities in the EEG rhythm (delta, theta and alpha waves). EEG recording seems to be useful for measuring stress levels and for predicting abnormalities due to different stress durations.

Keywords: Stress; Electroencephalogram (EEG); Adrenal gland; Rat

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Introduction

Stress is one of the main factors that highly influences physiological responsiveness and brain functions through many biochemical, hormonal, physiological and behavioral reactions (Radahmadi et al., 2015b). Indeed, psychical stress is known as a psychological and/or emotional stress that causes major health problems in human communities (Radahmadi et al., 2017). Also, stress can change such brain electrical activities as long-term potentiation and alterations in the electroencephalogram (EEG) rhythms (Loganathan and Rathinasamy, 2016; Radahmadi et al., 2015b; Seo and Lee, 2010). Indeed, EEG is an electrophysiological monitoring method to record the electrical activity in the cortex at multiple recording sites (Andrzejak et al., 2001). This makes EEG waves especially useful for studying memory...
imperfections and brain dysfunctions (Hanslmayr et al., 2011; Uhlhaas and Singer, 2006). Moreover, EEG abnormalities have been correlated with certain mental diseases (Freedman, 2003). EEG, thus, forms the golden standard for the early diagnosis of different mental disorders such as learning and memory deficits (Basar and Guntekin, 2012; Knyazev et al., 2006), sleeping disorder (Tang et al., 2007), anxiety and depression (Blackhart et al., 2006), epilepsy and schizophrenia among others (Ankanoglu, 2011; Moore et al., 1997; Peng et al., 2013). Moreover, it seems that EEG helps in the prediction of future diseases stemming from psychical stress.

Most people nowadays suffer from psychical stress due to their modern lifestyle. Stress is reported to be one of the major factors contributing to enhanced risks of disorders of one type or another (Reisi et al., 2017). This means that there is a close relationship between psychical stress and perturbations in mental health. For instance, stress causes imbalances in both sympathetic and parasympathetic responses in both the autonomous nervous system (ANS) and the hypothalamus-pituitary-adrenal (HPA) axis (Radahmadi et al., 2015b; Seo and Lee, 2010) and thereby, cause stress-related health problems (Seo and Lee, 2010). EEG signals can be effectively influenced by changes in ANS and HPA (Jena, 2015). Thus, the intensity and the patterns of brain electrical activity depend largely on the level of brain stimulation by different conditions and psychical stress durations. Despite the extensive research reported on the different aspects of stress, no published report is yet available on the effects of stress duration on variations in EEG waves and rhythms. The present study was, therefore, designed to investigate the effects of varying (acute, sub-chronic and chronic) stress durations on EEG waves and on adrenal gland weight as indices of stress.

Materials and methods

Experimental animals
For the purposes of this experiment, twenty-eight adult male Wistar rats weighing 250–300 g were procured from Pasteur Institute (Tehran, Iran). The rats were maintained under 12-h light/dark cycles (lights on 07:00–19:00) under controlled conditions of temperature (22±2°C) and humidity (50±5%). Food and water were made available ad libitum, except during the stress sessions. The experiments complied with the standards of the Ethics Committee of Isfahan University of Medical Sciences and were implemented in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23, revised in 1996).

The animals were randomly assigned to four groups (n=7 in each group) and the treatments included: one control and three experimental ones of acute, sub-chronic and chronic stress durations. The restraint stress was applied 6 hours/day for 1, 7 and 21 days in the experimental stressed groups. Finally, all the rats were prepared for EEG recordings on day 22.

Experimental procedures

Stress paradigms
The rats were placed in Plexiglas cylindrical restrainers (Razi Rad Co., Tehran, Iran) for 6 h/day (0800–1400) and fitted tightly (not allowed to move or turn around) over the stress session in each treatment (Radahmadi et al., 2015a). Restraint stress was defined as the situation involving both physical and psychological stress types (Avishai-Eliner et al., 2001; Nagata et al., 2009; Radahmadi et al., 2013, 2015b; Ranjbar et al., 2017). The situation was a strongly emotional stressor for the rats as it evoked unconditioned and unavoidable neuroendocrine responses (Radahmadi et al., 2015b). At the end of the stress sessions, the animals would be returned to their home cage.

Stereotaxic surgery and EEG electrophysiological study
Twenty-four hours after exposure to the last stress session (on day 22), the rats were initially anesthetized with intraperitoneal injections of urethane (1.5 g/kg; Sigma, USA) before being placed in a stereotaxic frame (Stoelting Co., USA). The skull was then exposed using a standard miniature drill equipped with a 0.5 mm diameter drill bit to drill two small holes on the frontal/parietal cortex for reference screw and the recording electrode. A reference stainless steel screw was placed over the nasal sinus in the frontal cortex (AP=1.7–2.2, ML= 2 mm from the bregma). A unipolar electrode (Teflon-coated stainless steel 0.125 mm in diameter; Advent Co., UK) was slowly lowered (about 0.2 mm/min) into the
The EEG activity of each anesthetized rat was recorded for approximately 20 min. Signals were low-pass filtered at 0.5–3 kHz and sampled at 1 kHz. They were additionally passed through an analog to digital interface (Data Acquisition Science Beam-D3111; eProbe- eTrace Experiment software) to a computer and the data thus obtained were analyzed using the eTrace Analysis software (Science Beam; Parto Danesh Co.). The delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz) and beta (13–30 Hz) waves were accepted (Jurkowlaniec et al., 2003). The total power of the four frequency bands were taken to be 100% as the base line, and the quantity of each frequency band (alpha, beta, theta and delta) as the percentage of the base line was calculated for all the groups examined (Miki Stein et al., 2017).

All the experiments were performed at the same time of the day (8–12:00 am) to avoid bias from the circadian rhythms. Finally, the brain was removed and stored in 10% formalin for at least 3 days. Frozen serial transverse sections (60 μm in size) of the brain were cut. The position of the electrode was determined according to a rat brain atlas using a light microscope (Fig. 1).

Adrenal gland weight assessment
The adrenal gland, considered in this experiment as a stress index, is the organ producing responses to stressful situations. At the end of the experiments (day 22), the adrenal glands were removed and weighed (Khasina et al., 1985; Ranjbar et al., 2017; Ulrich-Lai et al., 2006).

Data analysis
All the data were reported as means±SEM. The data obtained (between group comparisons) were
compared using ANOVA followed by LSD post-hoc test for multiple comparisons. A P-value of less than 0.05 was considered as statistically significant. Ultimately, the calculations were performed using SPSS 21 (SPSS Inc., Chicago, IL, USA).

Results

Figure 2 shows the effects of different stress durations on rat EEG traces. Figures 3 to 6 depict the responses of the different EEG brain waves in the parietal cortex over the CA1 region.

Based on the ANOVA test, the percentage of delta frequency showed a significant (P<0.05) enhancement in the chronic stress group as compared to the control (Fig. 3). This is while this parameter in the acute and sub-chronic stress groups did not exhibit any significant differences from those of the control (Fig. 3).

The percentage of theta frequency in the chronic stress group showed significant enhancements when compared with the control (P<0.05), acute (P<0.05) and sub-chronic (P<0.01) stress groups (Fig. 4).

As shown in Figure 5, the chronic stress group recorded significantly (P<0.05) higher percentages of alpha frequency than that recorded for the control. However, the alpha frequencies recorded for the acute and sub-chronic stress groups were not significantly different from those of the control (Fig. 5).

The percentages of beta frequency significantly increased in the acute stress and sub-chronic stress
Fig. 5. Comparison of percentages of alpha frequency (%) in the parietal cortex over the CA1 region in all the groups. Data represent mean±SEM (One-way ANOVA followed by LSD post-hoc test). *P<0.05 compared to the control group.

Fig. 6. Comparison of percentages of beta frequency (%) in the parietal cortex over the CA1 region in all the groups. Data represent mean±SEM (One-way ANOVA followed by LSD post-hoc test). *P<0.05 compared to the control group.

Fig. 7. Comparison of adrenal weight means in all the groups. Data represent mean±SEM (One-way ANOVA followed by the LSD post-hoc test). ***P<0.001 compared to the control group; #P<0.05 and ##P<0.01 compared to the acute stress group.
groups compared to the control ($P<0.05$, in both) (Fig. 6). The chronic stress group, however, showed no significant differences in this parameter from those of the control.

**Adrenal gland weight**

Following the decapitation of animals, adrenal glands were removed and weighed. Compared with the control group, the sub-chronic and chronic stress groups showed significant ($P<0.001$) increases in their adrenal gland weights (Fig. 7). The values for parameter also showed significant ($P<0.01$ and $P<0.05$) enhancements in the sub-chronic and chronic stress groups, respectively, as compared with the acute stress group, suggesting that the 7-day and 21-day stress periods might have stimulated adrenal activity under stress conditions (Fig. 7). The chronic stress group, however, exhibited a significant decline in adrenal gland weight when compared with the sub-chronic stress group (Fig. 7).

**Discussion**

The effects of acute, sub-chronic and chronic psychical stress durations were investigated on the percentages of alpha, beta, delta and theta frequencies in the EEG recordings taken from the parietal cortex over the CA1 region of rats in order to measure the levels of stress inflicted and to predict the likely abnormalities and disorders resulting from different stress durations. It was found that stress led to changes of EEG activity as revealed in the four brain waves examined (Fig. 2) although the different waves (alpha, beta, theta and delta) showed activity patterns varying in proportion to the different stress durations exercised. Moreover, chronic stress was found to cause changes in the alpha, theta and delta waves in the EEG recordings, but had no effect on the beta waves. Acute and sub-chronic stress durations changed only the beta waves (Figs. 3–6). While Mrdalj et al. (2013) reported lower EEG activities under stress conditions and no clear alterations in wave types. Our findings confirmed those of Jena (2015) who reported that animals subjected to mild and moderate stresses showed pronounced alpha waves recording but those subjected to high and severe stresses exhibited pronounced beta waves in their EEG recordings. Based on these observations, the sub-chronic stress (7-day stress) in the present study may be regarded as a high and severe psychical stress. This is further confirmed by the increased adrenal gland weight (as a stress index) (Fig. 6). It should be noted that both our previous and current studies indicate that changes in adrenal weight and corticosterone (CORT) level are positively correlated (Ranjbar et al., 2016). In the present study, beta waves increased in subjects exposed to acute and sub-chronic stresses whereas adrenal gland weight and CORT level increased in the sub-chronic and chronic stress groups in both our current and previous experiments. It, therefore, seems that although adrenal gland weight and CORT levels in the chronic stress group were lower than those in the sub-chronic stress one (Ranjbar et al., 2016), exhausting situation and adaptation failure occurred in the chronic stress group (Ranjbar et al., 2016; Ranjbar et al., 2017). On the other hand, according to Jena’s report on beta waves, acute (1-day) stress is a type of high stress. This is while, in present and our previous studies, we have shown that adrenal weight and CORT level do not increase in response to 1-day stress (Ranjbar et al., 2016) and the beta wave alteration in the acute stress group might have occurred due to the monoaminergic system (such as adrenalin) activities in response to acute stress. Our present results might, thus, be claimed to serve as an extension to our previous ones demonstrating that the 7-day stress experiment caused a high stress level in the individual and induced changes in brain activity as a result of adrenal activity and CORT secretion (Ranjbar et al., 2016). Moreover, previous studies have indicated that impairment of behavior under stress characterized by changes in brain functions (Jena, 2015; Ranjbar et al., 2016; Ranjbar et al., 2017) are due to the arousal of stress systems in the body (Radahmadi et al., 2015b; Seo and Lee, 2010). It seems that the different mechanisms involved in the induction of various EEG waves produce changes in the secretion of such neurotransmitters and hormones as cortisol (Heim and Nemeroff, 2002; Seo and Lee, 2010). Hence, some studies demonstrated that increased cortical activity probably was due to alterations in the secretion of acetylcholine and serotonin in the neocortex (Dringenberg et al., 2002; Lagopoulos et al., 2009). This cannot be verified, however, as we did not measure neurotransmitter levels in the present study. However, contrary to our
findings, Loganathan and Rathinasamy (2016) reported that noise stress did not cause any marked differences in plasma CORT or any significant changes in the EEG recorded of both prefrontal and occipital region. Moreover, the unique effects of stress on brain functions depend on the intensity, duration, type and nature of stressors (Radahmadi et al., 2015b).

It was the hypothesis of the current study that different durations of emotional stress should cause changes in brain waves and lead to different mental disorders. Our previous findings indicated that acute and sub-chronic stress (as shorter periods of stress) probably lead to elevated anxiety, decreased memory and to decreased cortical activity as evidenced by the subjects’ inability to cope with long stress durations (Ranjbar et al., 2015; Ranjbar et al., 2016; Ranjbar et al., 2017). This is in contrast to findings reported elsewhere to the effect that beta frequencies exhibit a desynchronized pattern during stress, strong excited emotions (e.g., fear) and states of consciousness (Jena, 2015). On the other hand, we observed a significantly enhanced alpha activity in the EEG recordings from the chronic stress subjects, again in contrast to Jena’s finding of an alpha activity pattern in relaxed and non-stressful conditions (Jena, 2015). It, therefore, seems that the chronic stress group adapted and coped with longer stress durations despite Knyazev et al.’s report that alpha waves were enhanced in anxious individuals (Knyazev et al., 2006). This difference may be due to the different methodologies employed such as EEG analysis. It has also been reported that alpha waves decreased and increased in schizophrenia and cognitive memory impairments, respectively (Basar and Guntekin, 2012; Moore et al., 1997).

Others observed theta waves to change significantly in chronic stress subjects who exhibited frustration and impaired information processing (Schacter, 1977). It has been reported elsewhere that cognitive and brain physiological processes are affected by chronic stress (Radahmadi et al., 2015b) and that they, in turn, affect electrophysiological characteristics, morphology and functions (Magariños et al., 1997; Radahmadi et al., 2014).

In general, although the mechanisms underlying EEG generation is not fully understood, interactions between various brain regions and cortical networks are assumed to play a key role in various rhythmical EEG activities under different stress durations.

**Conclusion**

In sum, the present study demonstrated consistent relationships among the brain bioelectric activity (EEG rhythm) and different (acute, sub-chronic and chronic) stress durations. Acute and sub-chronic stress durations led to changed cortical activity, indicating the inability of the subjects to cope with the stress imposed. Also, chronic stress caused irregularities in the EEG rhythm (delta, theta and alpha waves). Finally, EEG recording was found to be a useful means of measuring stress levels so that it can be used for predicting various abnormalities due to stress durations.

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**Conflict of interest**

The authors declare that they don’t have any conflict of interest.

**References**


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