Original article:

'Effect of Subacute Restraint Stress on mice in various Neurobehavioral Parameters'

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ABSTRACT:

Introduction: Stress is an important factor of depression that causes the changes in various body systems. Animal health including human has been shown to be affected by the stressful events of life inducing situation which alters cognition, learning, memory and emotional responses, causing mental disorders like depression and anxiety.

Aim & Objectives: The study was undertaken to assess the various neurobehavioral changes in mice applying restraint stress in mice after exposure to restraint stress.

Methods: The experiment was carried out with 12 healthy male mice weighing about 25-35gms. The animals were randomly divided into two groups of six animals each. Group I-control and Group II – restraint stress group. The restraint stress group mice were placed in specially constructed restraint meshes for 6 hours (between 10.00 am - 04.00 pm) for 21 days. The neurobehavioral assessment were done after 21 days by using open field apparatus and elevated plus maze for evaluating locomotor activity, emotional and anxiety status in mice.

Results: After 21 days of restraint stress, there was a significant ($P<0.001$) decrease in ambulation, rearing and grooming, whereas the immobilization time was significantly ($P<0.001$) increased when compared with their normal controls. In elevated plus maze, time spent in open arm, number of entries in open arms and number of entries in closed arms was significantly ($P<0.01$) decreased when compared to control group.

Conclusion: Restraint stress produces an inescapable physical and mental stress to which adaptation is seldom exhibited but in our study there is significant alteration in neuro behavioral studies.

Key Words: Restraint stress, cognition, behavioral changes, open field apparatus.

INTRODUCTION

Stress has become undoubtedly an integral part of human life. Stressful events have a damaging effect on normal physiological functions leading to a variety of diseases. Many of the diseases of the modern life like hypertension, diabetes, behavioral disorders have been suggested as one of the many deteriorated effects of stress. Experimental models are required to better understand the progression of the diseases which elaborate new therapy. Exposure to stressful stimuli produces widespread physiological and behavioral effects in animals including man [1]. Stress is an important factor of depression that causes the changes in various body systems. Animal health including human has been shown to be affected by the stressful events of life inducing situation which alters cognition, learning, memory and emotional responses, causing mental disorders like depression and anxiety.

However, many of these effects were mediated by stress-induced neurochemical and hormonal abnormalities that are often associated with oxidative stress[2]. It was demonstrated that subacute immobilization stress increases infarct volume and worsens neurologic outcome by actions that include excitotoxicity and inflammatory mechanisms in the brain[3]. The resultant disturbances due to immobilization may vary depending upon type, intensity, and the duration of a particular stressor and

the strain and sex of a subject[4]. This model of subacute restraint stress may be useful in investigating the mechanisms linking stress and pain, emotional status and anxiety to assess the potential therapeutic efficacy of diseases by various behavioral models[5].The earlier findings have reported that chronic and acute restraint stress procedures alter some behavioral parameters in mice. There are very few studies on subacute restraint stress in mice on neurobehavioral parameters. Therefore, the present study was designed to investigate the neuro behavioral parameters after subacute restraint stress procedure.

MATERIALS & METHODS

Animals

Male Swiss Albino mice, 25 to 30 g, were taken up for the study. Rodent chow and tap water were freely available. Mice were kept in a temperature-controlled room under standard laboratory conditions, with a 12-h light/dark cycle (lights on at 7:00 AM). The present study was conducted in the Department of Physiology, Meenakshi Medical College, RI, & Hospital. Enathur, Kanchipuram. Institutional Animal Ethical Committee (IAEC) clearance from MAHER was taken before we start the experiment.

Behavioral Tests

Elevated Plus-Maze Test

The anxiety status of the animal was assessed by elevated plus maze (Pellow et al., 1986). The elevated plus-maze consisted of two open arms (30 × 5 cm) and two enclosed arms (30 × 5 × 15cm) that extended from a central platform (5 × 5 cm). The entire maze was elevated 40 cm above the floor. During the first 5 min of free exploration, the number of entries into and the time spent in open and closed arms were recorded. An entry was defined as all four paws in an arm.

Open-Field Test

Emotional status of the animal and locomotor activity of the mice (Saillenfaint & Vannier 1998) was assessed by the parameters like, Peripheral ambulation, Central ambulation, Rearing, Grooming, Immobilization, Defecation and Urination. The open field was made of white acrylic (50 × 50 cm) with 22-cm-high walls. The floor was divided into 16 squares by black parallel and intersecting lines. Mice were placed singly in one corner of the open field, and entry latency (i.e., time to enter the first adjacent square), ambulation, and rearing were observed during a 5-min test. The experimental room for behavioral testing was an isolated quiet room with dimly lit environment. All behavioral experiments were conducted by a human observer in a blind fashion.

Experimental design:

Study Groups:

Group – I - Control (n-6)

Group – II - Restraint stress group (n-6)

Total - 12 animals

STUDY PROCEDURE

The restraint stress group mice were placed in specially constructed restraint meshes for 6 hours (between 10.00 am – 04.00 pm) for 21 days. The neurobehavioral assessment were done by using open field apparatus and elevated plus maze in mice and was compared with their control group for evaluating locomotor activity, emotional and anxiety status in mice.

The statistical analysis was done by using student's t - Test.

RESULTS

Fig 1: Comparision of behavioural changes in open field apparatus

In restraint stress subjected mice, locomotor activity and emotional status of the animal was decreased significantly as compared with normal control mice ($p<0.05$) in peripheral, central ambulations and in rearing. On the other hand in grooming, immobilization, defecation and urination the locomotor activity and emotional status of the animal increased significantly with normal control mice ($p< 0.05$).

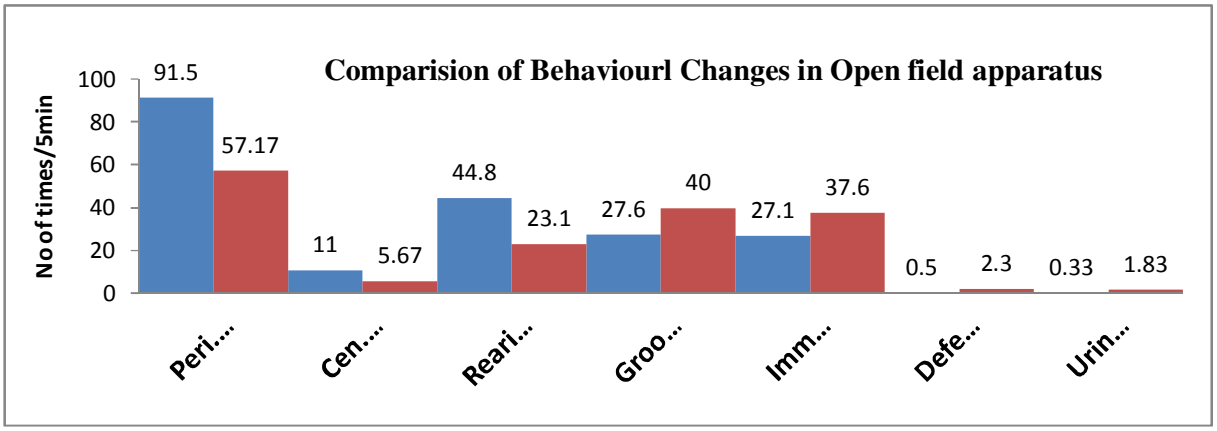
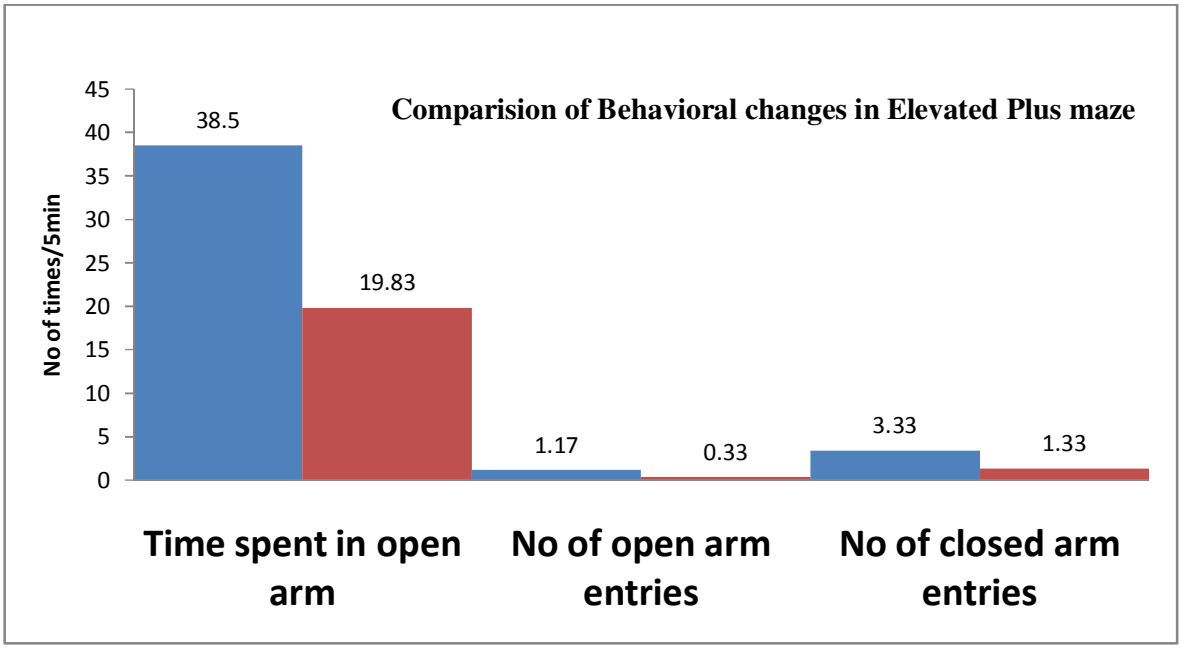


Fig 2: Comparision of behavioural changes in elevated plus- maze

In restrain stress subjected mice, anxiety status of the animal was decreased significantly as compared with normal control mice ($p<0.05$), was observed by time spent in open arm, number of open arm entries and number of closed arm entries.



DISCUSSION

The stress response in mice involves the perception and central processing of a stressor, followed by the release of corticotropin releasing hormone (CRH) from the hypothalamus, subsequent release of adrenocorticotropic hormone (ACTH) from the anterior pituitary, which causes the secretion of corticosterone from the adrenal cortex[6]. Corticosterone possess broad spectrum of actions affecting expression and regulation of genes throughout the body preparing the organism for changes in energy and metabolism for coping[7]. Stress has been postulated to be involved in the etiopathogenesis of a variety of diseases including hypertension, coronary heart disease[8], gastric ulcers[9], diabetes[10], immuno supression[11], mental depression, memory loss[12], and other diseases. The disturbances resulted by the stress may vary depending upon type, intensity, and the duration of a particular stressor and the strain\sex of a particular stressor differentiation of the subjects[13]. The exact mechanism of generation and progression of stress response is still unknown[14]. Immobilization by restraint stress is an aversive stimulus which disturbs physiological homeostasis and is reflected on a variety of biological systems of the body[15]. Exposure of animals to restraint stress and psychosocial stress in human implicated in pathophysiology of anxiety and mood disorders[16]. Our data reported that subacute immobilization stress in mice worsens animal behaviour through inflammatory mechanisms in brain causes altered neuro behavioral changes[17]. McLeod TM et *al., (*2001) reported that restraint stress causes wide range of anxiety like behaviour which alters cognition, learning, memory and emotional responses[18]. Caso JR *et al.,* (2008) reported that stress, either emotional or social, has damaging effects on cellular integrity in tissues particularly brain[19]. The present data reveals that, subacute restraint stress may affect the locomotor activity and increase the anxiety like behaviour. It may be due to, Immobilization by restraint stress is one of the explored models of stress that combined with emotional stress [escape reaction], Physical stress, resulting in both restricted mobility and aggression[20]. Physiological response to stress is the activation of the HPA axis and causes release of corticosterone, which in turn accelerate the generation of free radicals[21,22,23,24]

CONCLUSION

Restraint stress produces an inescapable physical and mental stress to which adaptation is seldom exhibited but in our study there is significant alteration in neuro behavioral studies. The stress procedure affected the locomotor activity and altered behavioral changes (emotions and anxiety) in open field behavioral model and elevated plus maze.

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