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Research paper

Chronic exposure to immobilized stress induces memory impairment and produces other behavioral deficits in male rats

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ABSTRACT

Stress is one of the leading disorders in today's world. It is the body's natural response against uncertain circumstances that is produced in response to a stimulus that can be physiological or neurochemical. Immobilized stress is the condition in which the body is placed under a physical or physiological stressor that results in inducing changes in the body's normal state. In the present study, rats were treated with immobilized stress. 12 locally bred Albino Wistar rats were taken and categorized into 2 groups; control and test. Immobilized stress was given using restrainers. Behavioral tests were performed to see the effect of immobilized stress; the Morris water maze test was used to evaluate the working memory functions, elevated plus maze test for anxiety-like effects and depression-like symptoms was assessed by using the force swimming test. Results indicated that the immobilized stress to test animals leads to the impairment of working memory functions; simultaneously anxiety was also induced in the immobilized animals. Results also showed immobilized stress-induced depression-like symptoms. In conclusion, immobilized stress exhibits memory impairment which indicates that immobilized stress results in other substantial behavioral deficits in rats.

KEYWORDS Stress, Morris water maze, Memory, Depression

INTRODUCTION

Stress produced a significant effect on human health, causing several different several psychological problems [1]and pathological conditions [2]. Studies suggested that any stressor either external or internal can trigger psychological and physiological responses in organisms [3]. It is well-known to contribute to behavioral deficits like cognitive, anxiety, and physiological variations regarding heart functions [4]. It has been studied that immobilized stress is a psychological stressor that, like conditioned fear, leads to the secretion of ACTH and corticosterone [5]. Restraint is a commonly used psychological stressor [6]. Chronic restraint stress causes modification and reversal of hippocampus functions.

Different studies suggested that brain areas especially the amygdala and hippocampus involved in the stress response. Abnormalities in current areas may lead to the development of post-traumatic stress disorders [7]. Omar with coworkers (2017) reported that some neurotransmitter deficiency like serotonin and catecholamine is associated with anxiety and other mood disorders [8]. Scientists suggested that serotonin and the hypothalamic–pituitary–adrenal axis are closely related and possess a major contribution to normalizing circadian rhythm and stress. Moreover, their performance helps in minimizing several neurological disorders like depression, and anxiety [9]. Grant *et al*. (2017) [10] suggested that short-term stress can widely affect memory functions. The present study is designed to characterize the effects of immobilized stress on behavioral deficits in rats.

MATERIALS AND METHODS

Bacterial strain and growth conditions

12 albino Wister rats were used in this study. They were all locally bred (150-200 g) obtained from Aga Khan University Hospital. After bringing they were caged separately in a quiet room under a 12 h light-dark cycle (light on at 6:00 h) and maintained surrounding temperature (22℃ ± 2). Initially, animals were habituated at least 3 days before experimentation. All experiments were approved by the institutional review board (IRB) of Federal Urdu University, Karachi, Pakistan.

Behavioral Tests

Restrain Stress

For Immobilized stress, animals were daily restrained for 3 hrs. The procedure was repeated continuously for about 15 days in well-ventilated Plexiglas tubes. During stress, they have no access to food and water.

Morris Water Maze Test

Working memory functions in animals were examined by using Morris water maze apparatus. This apparatus used in the study comprised of a circular tank which is of transparent glass. Liquid in the tank is opaque with a hidden platform. Experimentation and specifications of Morris water maze test was as according to Haider *et al* (2016) [11].

Forced Swimming Test:

This apparatus is designed in a glass tank. Specifications and experimentation is explained [11]. During the testing phase swimming behavior of animals were observed in which animal force to swim throughout the swim chamber. Experiment is based on monitoring of the immobility time. So when the animal is showing immobile status it will only keep its head above the water and attempting no efforts of swim.

Elevated plus Maze Test

Elevated plus maze test is commonly used to assess anxiety functions [4]. Design of this apparatus also helps in assessing memory functions. However in this experiment it is used to see anxiety like effects. Detail experimentation is described in [11]. Rats placed at end of open arm and time taken to enter in close arm is noted.

Statistical Analysis

Behavioral data were analyzed by using independent sample t-test by SPSS version 20. p values<0.05 were considered significant. Values in graph are representing as means ± SD (n=12).

RESULTS

Figure 1 shows the effect of repeated exposure of immobilized stress on food intake in animals. Data analyzed by student's t-test revealed significant decreased in food intake due to exposure to immobilized stress ($t= 6.2238$, df: 10, $p< 0.05$). Figure 2 indicates the effect of repeated exposure of immobilized stress on memory functions in rats. Analysis of data by student's t-test revealed a significant effect of stress ($t=4.228$, df: 10, $p< 0.01$) on long term memory functions. Results show that immobilized stress in rats possess a significant decrease in ($p<0.01$) memory functions as not in the unstressed control rats. Immobilized stress on depression like symptoms in rats show in figure 3. Data showed a significant effect of restrain stress treatment ($t=3.3822$, df: 10, $p<0.01$). Immobility time of stressed rats were significantly increased ($p<0.01$) as compared to control rats. In figure 4 the effect of immobilized stress on anxiety in rats using elevated plus maze apparatus. Data represented a significant treatment effect of stress exposure ($t= 4.1265$, df: 10, $p<0.01$) on anxiety like effects. The results

show that control rats possess less anxiety like effects as compare to stressed animals.

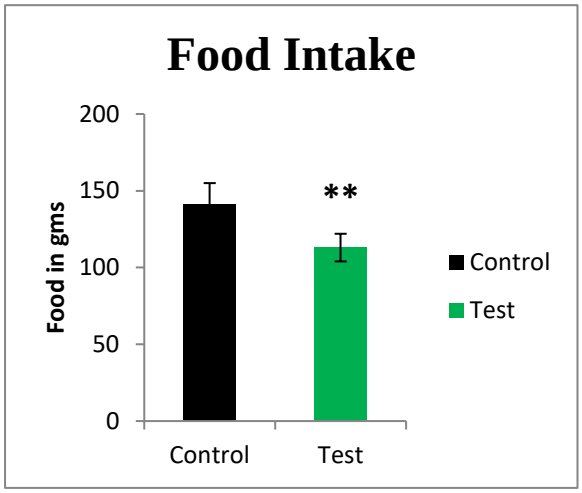

Figure 1: Effect of immobilized stress on feeding behavior in rats. Significant difference by student's t-test: **P<0.01 compared with controls.

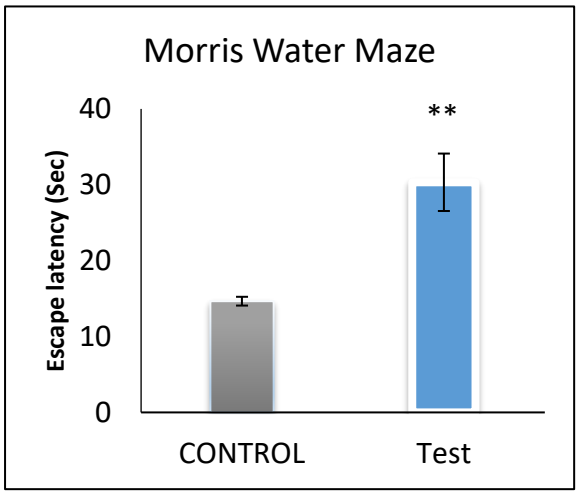

Figure 2: Effect of immobilized stress on working memory functions in animals. **P<0.01 compared with controls showing significant difference by student's t-test method.

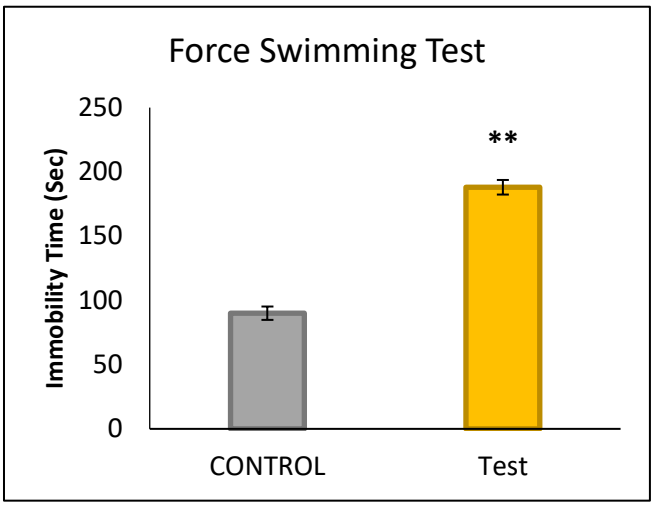

Figure 3: Effect of immobilized stress on depression like symptoms in rats. **P<0.01 compared with controls showing significant difference by student's t-test method.

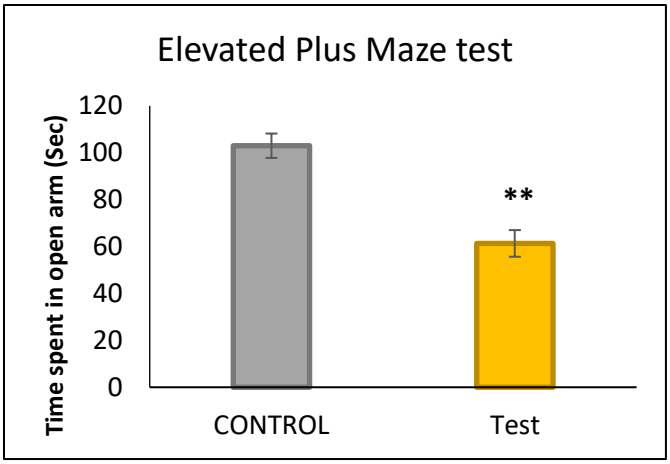

Figure 4: Effect of immobilized stress on working memory functions in animals. **P<0.01 compared with controls showing significant difference by student's t-test method.

DISCUSSION

Present study revealed that 15 days immobilized stress impaired memory functions and produces depression like symptoms. Exposure to stress leads to the anxiety in animals and effects on food intake. Bloss et al [12] explained that immobilization stress in animals leads to the decreased weight gain as compare to unstressed animals. This decreased body

weight may be attributed to the minimized food intake in the animals in response to stress exposure. Moreover, this decrease in the body weight can also have association with altered adrenal secretion, maldigestion and increase in metabolic demands following stress treatment [12]. In our study animals exposed to restrainers showed decreased food intake which may leads to decreased gain in body weight. Present study can correlate with the effect on the feeding center controlled by brain as a result animal food intake gets significantly reduced.

Current study shows that chronic exposure to immobilized stress impairs memory functions. Previous researches showed that chronic exposure to stress effected on cognition and mental health which may lead to change in brain structures [13]. Nicola et al. [14] proposed that depression and anxiety like function are associated with the cognitive dysfunctions due to excess in glucocorticoids levels after exposing to stress condition. Inoue et al [15] explain that stress induced the glucocorticoid receptors and results in increased concentration of glucocorticoid which is associated with memory disruption. Previous studies also explained that different brain regions like limbic regions effected due to stress and impact on neurotransmitters as well as some hormones which indicates the involvement of the HPA axis in these events [16]. This mechanism supported our results also that shows decrease latency time tested in Morris water maze test.

Chronic immobilized stress showed increase in depression like symptoms as well as producing anxiety like effects. Patki et al. [17] proposed that rats after socially defeated possess anxiety-like functions and cognitive problems arise due to social defeat. Long term exposure to stress increases fear conditions anxiety and other mood. Research reported significant effects of immobilized stress on aggression, inhibitory avoidance, escape, as well as learned aspects of fear and inescapable stress observed [18]. Our result also showed that immobilized stress leads to the depression like symptoms and produces anxiety like effects in animals. Jason et al. [19] proposed that chronic exposure to immobilized stress dysregulate the glucocorticoids and this mal regulation is strongly associated with cognitive function and depressive illnesses. Our current study is also associated with the depression and anxiety like effects due to chronic exposure to stress. Further Jason et al. [19] explained that depression and anxiety associated with decreased neurogenesis. Hyeonwi et al [20] also hypothesized that long term exposure to immobilized stress validate depression like symptoms in individuals.

CONCLUSION

In conclusion repeated exposure to immobilized stress produces memory impairment and other behavioral deficits. Memory impairment could be due to HPA axis stimulations and immobilized stress produces depression like symptoms and anxiety that result in decrease food intake and may effecting physiological functions. Further studies needed to confirm biochemical and neurochemical basis following immobilized stress.

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