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Original research article

Effect of forced swim stress on wistar albino rats in various behavioral parameters

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Abstract:

Introduction: Stress is an important factor of depression that causes the changes in various body systems. The forced swim test is a commonly used stressor test where rats are forced to swim in specially constructed tanks for a particular period where there is behavioral activation characterized by vigorous swimming and diving to search for alternate routes of escape. 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 and stress in rats. **Methods:** The experiment was carried out with 12 healthy albino Wistar female rats weighing about 150-180gms. The animals were randomly divided into two groups of six animals each. Group – I (control), Group – II (Stressed Group). Group –II rats are placed in plastic tanks for 45minutes for15 days. Temperature of water was maintained at 20˚C. During stress phase, the animals will be trained for forced swim test, behavioral changes observed by open field apparatus for emotions, and eight arm maze for memory & leaning, elevated plus maze for anxiety. **Results:** Forced swim stress causes to a significant change ($p<0.05$) on cognitive functions: motivation, learning and memory. Forced swim stress is the factor damaging the hippocampus causes repeated immobilization and produce atrophy of dendrites of pyramidal neurons and neuroendocrinological disturbances, controlled by the hypothalamo-pituitary-adrenal axis (HPA). Repeated stress in the form of forced swimming activates the free radical processes leading to an increase in lipid peroxidation in many tissues. **Conclusion:** This study reveals the effect of repeated forced swim stress causes wide range of adaptive changes in the central nervous system including the elevation of serotonin (5-HT) metabolism and an increased susceptibility to affective disorders. The earlier findings have reported that chronic and acute stress procedures weaken working memory functions in rats. There are very few studies on sub acute forced swim stress on working memory status of the animal. The study was undertaken to assess the various behavioral changes by applying sub acute forced swim.

Key Words: Forced swim stress, Cognition, Behavioral changes, Open field apparatus.

Introduction

Stressful situations induce physiological and behavioral changes in an organism to maintain the homeostasis. Exposure to stressful situations is among the most common human experiences. In response to stressors, a series of behavioral, neuro chemical, and immunological changes occur that ought to serve in an adaptive capacity[1]. Swimming in small laboratory animals has been widely used for studying the physiological changes and the capacity of the organism in response to stress[2]. Swimming is not always a simple exercise stress, because emotional factors are difficult to be eliminated[3]. The forced swimming stress developed by Porsolt et al. (1977) has now become widely accepted model for studying physical stress in animals. Water temperature is another important factor in forced swimming test. By varying the water temperature, Richter (1957) found that rats could survive as long as 80 hours in lukewarm water (36° C). Increasing or decreasing the water temperature above or below this point influences the overall behavior of the animal and changes the involvement of glucocorticoids[4]. The conversion of oxygen during normal metabolism to the byproducts, hydrogen peroxide, Super oxide and hydroxyl radical occurs by successive electron additions to oxygen[5]. Toxic free radicals have been implicated as important pathological factors in cardiovascular diseases, pulmonary disorders, autoimmune diseases, cancer, metabolic disorders, and aging[6]. However, swimming has also been used to elicit stress responses in rats[7]. This stress is usually elicited by an acute forced swim session of between a few minutes to half an hour[8]. Psychological stress (e.g. foot shock, forced swim test) raises levels of corticosterone in the rat[9]. Which in turn leads to physiological changes that include increased myocardial infarct size[10]. Increased memory loss and cognition[11,12]. Thus we set out to determine whether forced swim training had an effect on corticosterone levels or elicited a change in behavior of rats in the open field.

Materials and Methods: Animal model: Adult female Wistar rats weighing between 150-250 g were divided into two groups as Control (n =12) and Stress (n =12). All the rats were given standard rat chow and tap water *ad libitum* and were housed at 25 ± 2 o C on a 12-hour dark/light cycle. All the experimental procedures were approved by the IAEC; adequate measures were taken to minimize pain or discomfort.

Stress procedures: Rats were exposed to forced swimming stress daily for duration of 45 minutes between 09.00AM to11.00AM until 21 days. They were forced to swim in plastic tanks (length 100cm, width 40 cm, depth 60 cm) containing tap water maintained at a temperature of 20° C. The depth of water in the tank was 30 cm. A maximum of two rats were allowed to swim together during stress session. The control rats were housed under the same conditions and they were handled as often the stressed group.

Behavioral studies: Open-field apparatus (OFT)

The open field test is a common measure of exploratory behavior both qualitatively and quantitatively. Each rat was placed in the open field for 5 minutes to test for differences in anxious-like behavior and activity. This was done 24 hours after the last bout of swimming exercise. The open field test is designed to measure behavioral responses such as locomotor activity, hyperactivity, and exploratory behavior. The open field is also used as a measure of anxiety. Rats tend to avoid brightly illuminated, novel, open spaces. The apparatus for the open field test is a square enclosure (1 m by 1 m) made of black Perspex. To analyze exploratory and locomotor activities as an indication of stress in the rat, animals were placed in the left rear quadrant of an open field. The number of line crossings and the total distance covered by the rat were measured over 5 minutes. These are classical measures of locomotor and exploratory activities. The more time the rat spends in the inner zone of the open field, and the more exploratory the rat is, the less stressed it is perceived to be.

Procedure: Each rat was placed individually in a corner of the field and its behavior recorded for 5 minutes. All activity was recorded using a video

camera mounted above the open field and scored later by an advanced motion-recognition software package (**Noldus Ethovision version 3.1 software**) that detects and analyzes the movements of the rat. The video image of the open field arena was partitioned into 36 equal-size squares; 24 border squares and 12 centre zone squares. Total distance, average speed, and time spent in various parts of the field (e.g. the border areas vs. the open, middle area) were measured and analyzed. Testing was carried out in a temperature, noise and light controlled room. During the test procedure silence was maintained in the test room. The behavioral tests were performed from 1-1:30 pm daily to ensure that normal daily fluctuations in corticosterone, circadian rhythm and activity did not affect the results. The rats were placed in a cage in the testing room an hour before the test in order for them to acclimatize to the new environment. The open field was cleaned with 70% ethanol after each rat had been tested. Each rat was tested individually and in a separate test room. Throughout the entire testing-session, the sequence of events and procedures was always the same and the test circumstances (handling, room-features, equipment used) were as standardized and controlled as possible. The entire test procedure lasted approx. 20 minutes per animal, and was recorded on videotape to allow analysis at a later time.

Elevated plus-maze apparatus (EPM): Elevated plus-maze is the simplest apparatus to study anxiolytic response of almost all type of anti anxiety agents. Exposure of the animals to novel maze alley evokes an approach avoidance conflict which is stronger in open arm as compared to enclosed arm. Rodents (rats and mice) have aversion for high and open space and prefer enclosed arm and, therefore, spend greater amount of time in enclosed arm. When animals enter open arm, they freeze, become immobile, defecate and show fear-like movements. The plasma cortisol level is also reported to be increased, as a true reflection of anxiety (Kulkarni et al., 2009). The elevated plus-maze was slightly modified from that used by Lister (Lister et al., 1987). Briefly, it consisted of two open arms (30 cm×5cm×0.25 cm) and two enclosed arms (30 cm× 5cm× 15 cm), extending from a central platform (5 cm× 5 cm) and raised 50 cm above floor level. The maze floor was constructed from black Plexiglas and the walls from clear Plexiglas. The conventional spatial–temporal measures recorded were the number of entries (all four paws on open or enclosed arms and expressed as percentage of total entries), the time spent on open arms (expressed as percentage of time spent on closed plus open arms), number of entries on enclosed arms and the time on the central platform. Ethologically derived measures were grooming, rearing, as an emotionally related parameter. A selective increase in the parameters of exploration of the open arms of the maze reveals an anxiolytic effect (Rodgers et al., 1992; Pellow et al., 1985).

Table.1: Effect of stress on various Behavioral Parameters in open filed apparatus

Parameters	Mean ± SD		**P value**
	Control	Stress Group	
Peripheral ambulation	74.5 ± 15.9	92 ± 11.7	**<0.05***
Central ambulation	13.8 ± 3.6	4.33 ± 1.86	**<0.001****
Rearing	44.17 ± 10.5	24.5 ± 7.61	**<0.005****
Grooming	31.5 ± 5.58	40 ± 9.05	**<0.005****
Immobilization	28.6 ± 5.82	40.6 ± 5.2	**<0.004****
Defecation	0.83 ± 0.41	1.83 ± 0.98	**<0.05***
Urination	**0.50 ± 0.55**	**2.17 ± 1.47**	**<0.03****

Table.2: Effect of stress on various Behavioral Parameters in Elevated plus maize

Parameters	Mean ± SD		P value
	Control	Stress Group	
Time spent in open arm	38.3 ± 5.72	31.5 ± 3.89	**<0.03****
Number of Open arm entries	1.67 ± 0.52	0.83 ± 0.75	**<0.05***
Number of Closed arm entries	2.67 ± 1.03	1.5 ± 0.84	**<0.05***

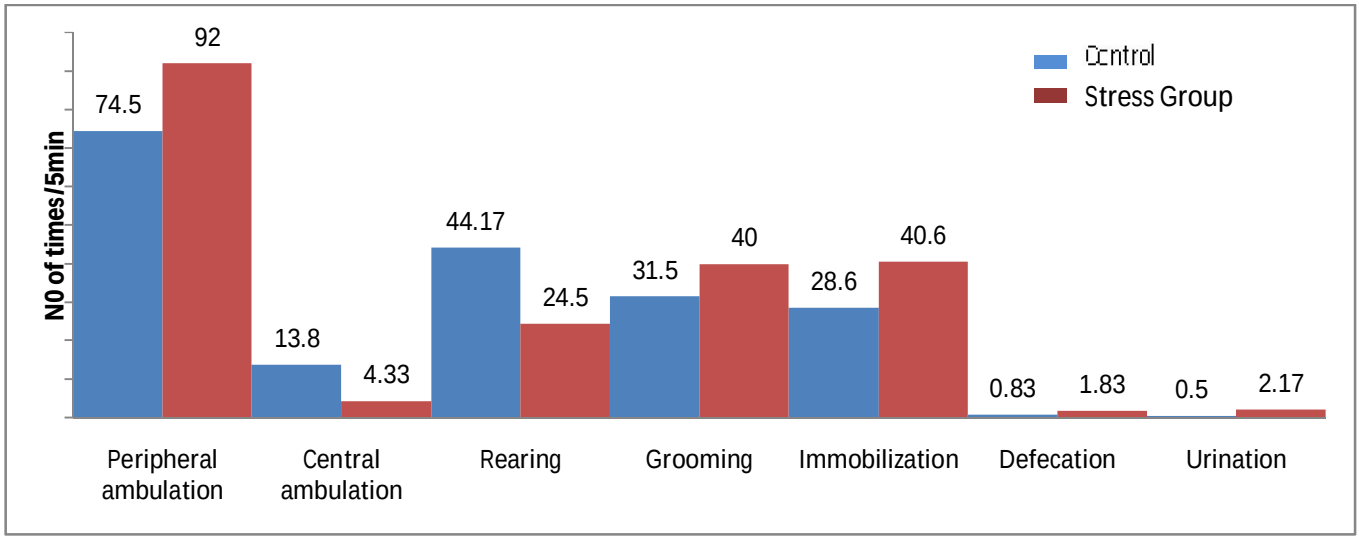

Fig. 1: Effect of stress on various Behavioral Parameters in open filed apparatus

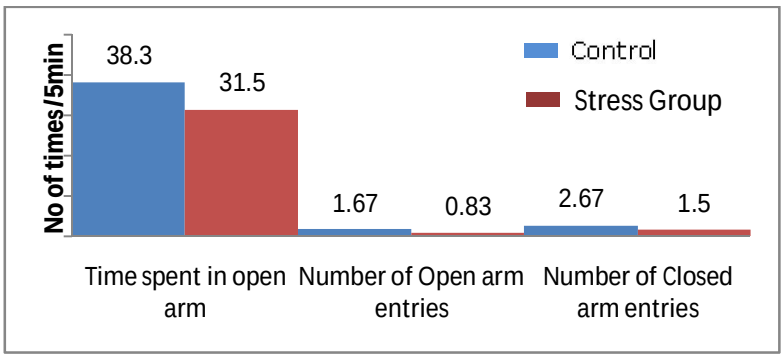

Fig. 2: Effect of stress on various Behavioral Parameters in Elevated plus maize

Discussion:

Forced swimming has been used to elicit stress response in rats[13]. Psychological stress like foot shock, forced swim test raises levels of corticosterone in the rats[14]. Corticosterone-releasing hormone is commonly released during stress and might be a factor that suppressed food appetite in the forced swimming stress. The importance of our study is evident that the neurons in the brain are exposed to pulsatile pattern of free corticosteron[15]. The forced swim test is used as a model of stress depression in neurological studies[16]. In stress linked neuropsychiatric disorders like recurrent depressive illness, there is evidence of

structural changes in the hippocampus, a brain region extensively studied with regard to stress[17]. The present data indicate that forced swimming stress a period of 21 days, 6 hour decreased the whole body weight and food intake and increased weights of liver, kidney and adrenal glands and exploratory behavior. This experiment indicate that 21 days of daily stress is associated with impaired acquisition and performance of a spatial memory task, the behavioral models suggest that the hippocampal atrophy present after the stress[18]. The present findings suggest a possible role for endogenous opiates in behavioral arousal[19]. Immobility time in the stress group was 6 hours/ day for 21 days significantly reduced the immobility time. These results indicate that the swimming stress caused a depressed state in the stressed group. Concerning neurotransmitters in the hypothalamus region, 5- HIAA/5-HT ratio significantly decreased in the stressed group due to increased 5-HT levels and decreased 5-HIAA levels compared to the rats without FST, suggesting that the decrease of the ratio possibly reflects the decline of 5-HT metabolic activity due to swimming stress. These phenomena may be included in key mechanisms of the development of depression**.** Forced swim stress causes to a significant change ($p<0.05$) on cognitive functions: Forced swim stress causes Peripheral Ambulation $P < 0.05$, Central Ambulation $P < 0.001$, Rearing $P < 0.005$, Grooming $P < 0.005$, repeated Immobilization $P <0.004$, Defecation $P< 0.05$, Urination $P < 0.03$, Time spent in open arm $P< 0.03$, No. of open arm entries $P < 0.05$ and No. of closed arm entries $P < 0.05$ and factor damaging the hippocampus causes produce atrophy of dendrites of pyramidal neurons and neuro endocrinological disturbances, controlled by the hypothalamo-pituitary-adrenal axis (HPA). Repeated stress in the form of forced swimming activates the free radical processes leading to an increase in lipid peroxidation in many tissues[20].

Conclusion: This study reveals the effect of repeated forced swim stress causes wide range of adaptive changes in the central nervous system including the elevation of serotonin (5-HT) metabolism and an increased susceptibility to affective disorders. The earlier findings have reported that chronic and acute stress procedures weaken working memory functions in rats. There are very few studies on sub acute forced swim stress on working memory status of the animal. The study was undertaken to assess the various behavioral changes by applying sub acute forced swim.

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