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Research article

A STUDY ON BEHAVIOURAL CHANGES INDUCED BY COLD WATER STRESS IN SWISS ALBINO MICE

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ABSTRACT

Introduction: Stressful condition induces physiological and behavioral changes in an organism to maintain the normal homeostasis. The response to stress is regarded as a positive adaptive process of an organism, which consist of a set of physiological and behavioral reactions to cope up with the challenging situations. In animals, stress study has shown to affect locomotor activities and behavioral changes. The present study was to determine the effect of cold water stress in different duration on neurobehavioral changes in swiss albino mice. **Material and Methods:** Male swiss albino mice weighing about 20 to 25gm were chosen for the study. The study consists of 4 groups (i) control, (ii) 1 day cold stress, (iii) 7 days cold stress and (iv) 14 days cold stress. The cold stress procedure was done by allowing the animal to swim in cold water at 14°C for 5minutes every day. After the stress period, the neurobehavioral parameters were studied by using open field behavior and elevated plus maze. **Results:** There was a significant decrease in ambulation, rearing, grooming with a significant ($P<0.001$) increase in immobilization time of 1 day cold stress exposed group and 7th day cold stress exposed group in open field behavior model, whereas the 14th day stress group showed a significant decrease in ambulation only. In elevated plus maze, time spent in open arm was significantly decreased in 1st and 7th day stress group ($P<0.001$) and the number of entries in open arms and closed arms was significantly decreased in all stress exposed groups when compared with their respective control groups. **Conclusion:** Cold stress can affect hypothalamic- pituitary adrenal axis and thereby increase anxiety resulting in change in behavior and locomotor activity. When the stress period was prolonged to 7-14 days, there was a gradual recovery back to near normal. This type of behavior of the animals to prolonged stress could be attributed to habituation of the hypothalamo-pituitary adrenal axis.

Keywords: Cold stress, open field behavior, elevated plus maze, and immobilization.

INTRODUCTION

Stressful condition induces physiological and behavioral changes in an organism to maintain the normal homeostasis. The response to the stress is regarded as a positive adaptive process

of an organism, which consist of a set of physiological and behavioral reactions to cope up with the challenging situations. In animals, stress study has shown to affect locomotor activity and behavioral changes along with exploratory behavior. The forced swimming stress developed by has now become widely accepted model for studying physical stress in animals[1]. In swimming stress, water temperature plays an important role. When the water temperature falls below 18^{o}C, it affects both the physical and mental task. Cold water stress affects the animal directly by cold exposure to the whole body at a time. By varying the water temperature, it is found that rats could survive as long as 80 hours in lukewarm water (36^{o}C)[2]. Any kinds of stress stimulus can results in a significant activation of the sympathoadrenal medullary system and a measurable change in the behavior. When the animal is subjected to acute stress, a wide range of physiological and behavioral changes takes place, chronic exposure results in to habituation or adaptation occurs, but it depends upon the intensity and duration of the stress. In all type of stress it has been observed that there is increase production of corticosterone in plasma and also it has been reported that exposure to stress situations can stimulate numerous pathways, leading to increased production of oxygen free radicals, free radicals generate a cascade producing lipid peroxidation. Lipid peroxidation is one of the main events induced by oxidative stress[7]. The present study is to determine the effect of cold water stress by different duration on neurobehavioral changes in Swiss albino mice.

MATERIALS AND METHODS

The study was approved by Institutional Animal Ethics Committee. Healthy adult male Swiss albino mice weighing (20-25gms) were used for the study. Mice were housed under standardized conditions with free access to food and water. Animal procedures were performed in accordance with the Ethics Committee. The study consist of four groups, Group I – control, Group II – 1 day cold stress, Group III – 7 days cold stress and Group IV – 14 days cold stress. The cold water stress procedure was done by allowing the animal to swim in bucket containing cold water at the depth of 20cm and the temperature was maintained at 14°C for 5 minutes[20].

Parameters : The neurobehavioral parameters were studied by using Open field behavior model (Saillen faint et al) and Elevated Plus Maze (Pellow et al). Exploratory and locomotor behavior was assessed by placing animals individually into an open-field box marked off into 25 equal squares. A mouse is placed on one corner of the apparatus and is observed for 5 minutes. The parameters noted are a) Ambulation b) Immobilization c) Rearing d) Grooming and e) Urination.

The anxiety of the animal was assessed by using elevated plus maze apparatus. The parameters noted are

a) Time spent in open arm, b) Number of open arm entries and c) Number of closed arm entries.

RESULTS

All data were analyzed by using student's t-test, There was a significant decrease in ambulation, rearing, grooming with a significant ($P<0.001$) increase in immobilization time of 1 day cold stress exposed group and 7th day cold stress exposed group in open field behavior model (table-1) , whereas the 14th day stress group showed a significant decrease in ambulation only. In elevated plus maze, time spent in open arm was significantly decreased (table-2) in 1st and 7th day stress group ($P<0.001$) and the number of entries in open arms and closed arms was significantly decreased in all stress exposed groups when compared with their respective control groups.

Table: 1 Behavioral study - Open-field test

Test name	Duration of stress	Mean ± S.D	P- value
Peripheral	1 day	44.17±13.63	0.0001*
ambulation	7 days	46.67±13.71	0.0001*
control : 91.5±5.43	14 days	68.00±11.80	0.0030*
Central	1 day	3.00±2.37	0.0001*
ambulation	7 days	4.17±2.40	0.0002*
control : 11.00±2.19	14 days	6.17±1.83	0.0021*
Rearing	1 day	28.50±5.43	0.0127*
(no of times/5min)	7 days	28.83±7.33	0.0160*
control : 44.83±10.93	14 days	35.00±9.38	0.1260
Grooming	1 day	18.83±5.46	0.0103*
(no of times/5min)	7 days	21.33±7.20	0.0480*
control : 27.67±3.88	14 days	25.17±10.32	0.5976
Immobilization	1 day	59.33±11.59	0.0004*
(no of times/5min)	7 days	59.00±8.81	0.0000*
control 27.17±7.31	14 days	35.67±17.40	0.3078

*** Significant P < 0.05**

Table: 2 Elevated plus mice

Test name	Duration of stress	Mean± S.D	P- value
Time spent in	1 day	9.83±7.31	0.0001*
open arm	7 days	12±6.9	0.0001*
control : 33.3±5.16	14 days	27.67±7.74	0.1709
No of open arm	1 day	0.5±0.55	0.0012*
entries	7 days	1.17±1.03	0.0100*
control : 3.33±1.21	14 days	1.5±1.05	0.0190*
No of closed arm	1 day	1±0.89	0.0001*
entries	7 days	1.17±0.75	0.0001*
control : 4.0±0.89	14 days	2.17±1.17	0.0131*

***Significant P < 0.05**

DISCUSSION

Cold water stress, as a natural stressor, may have its own unique pattern of neurobehavioural changes, results of the study provide the information that, cold water stress may affect the locomotor activity and increase the anxiety like behaviour, and when the stress period continues, there is recovery back to near normal, the probable reason could be as follows, Physiological response to stress is activation of the hypothalamic – pituitary - adrenal axis and subsequent release of corticosterone which in turn accelerate the generation of free radicals. Excessive production of free radicals resulted in oxidative stress, which leads to damage of

macromolecules and cause degeneration of tissues[7]. Excessive shivering due to cold stress contributes to fatigue and makes performance of motor skills more difficult and altered excitability of the nervous system[8]. Repeated stress on a daily basis may impair the antioxidant defenses in the body leading to oxidative damage by changing the balance between oxidant and antioxidant factors. The increased levels of reactive oxygen species under stress conditions could be due to the increased concentration of glucocorticoids. It has been reported that these hormones exacerbate reactive oxygen species (ROS) generation in the body[9]. Decreased activities of the antioxidant enzymes have been observed in the brain of rats treated with glucocorticoids[10]. An increase in lipid peroxidation indicates the increase in the free radical generation due to repeated stress and a decrease in resistance against stress. Increasing stress was reported to enhance stress-induced tissue damage and malfunction[11]. Oxidative stress is a central feature of many diseases and stress-induced damage to these tissues may be the cause of severe stress disorders after repeated stress exposure. Numerous studies have shown that strenuous physical exercise can increase free radical production and cause oxidative damage[12]. Apart from repeated swimming exercise, decreased water temperature also produced severe stress in rats and these two factors together might have caused increased free radical production in various tissues[13]. Brain serotonin is involved in mood disorders such as depression and anxiety as well as in nociception and thermoregulation[14]. The hyperactive serotonergic system causes anxiety like behavior in rodents and humans[15,16], the activation of the serotonergic system may thus contribute to the development of anxiety- related disorders. The cold stressed mice may thus be related to the serotonergic system. Cold stress was shown to decrease serotonin in various brain areas of mice such as the cerebral cortex, hypothalamus, thalamus and midbrain[17].

Forced swimming stress significantly lowered the serotonergic ratio and also markedly enhanced the phosphorylation of ERK1/2 (extracellular signal regulated kinase) in the hypothalamus region and this may be the key mechanism for the development of depression to the animal[18]. Repeated cold stress produces a specific pattern of changes in spontaneous activity and responses to sensory stimuli in lateral hypothalamic area (LHA) and medial hypothalamic area (MHA) neurons; this could underlie the behavioral changes induced by repeated cold stress such as hyperphagia and hyper-reactivity to sensory stimuli[19-21]. Behavior changes of the animals to prolonged stress could be attributed to habituation of the hypothalamic - pituitary-adrenal axis[7].

CONCLUSION

Cold stress affects the locomotor activity and altered the behavioral parameters in open field behavioral model and elevated plus mice. Cold stress affect hypothalamic- pituitary adrenal axis and when the stress period was prolonged to 7-14 days, there was a gradual recovery back to near normal, which shows the animal is getting adopted slowly. But the adaptation of the animal depends on the duration of stress period and intensity of stress.

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