

EFFECTS OF 2hrs. RESTRAINT STRESS ON BRAIN SEROTONIN METABOLISM AND MEMORY IN RATS

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ABSTRACT

Stress is a non-specific response of body to any physiological and psychological demand. Studies on experimental animals show that uncontrollable stress situation produce neurochemical and behavioral deficits

In the present study effects of 2hrs. restraint stress were monitored on brain serotonin metabolism and memory in starved rats. An episode of 2hrs. restraint stress exhibited a significant decrease in food intake as well as in growth rate in starved rats. The memory function was significantly enhanced following 2hrs. restraint stress in starved rats. The concentration of Noradrenaline (NA) and Dihydroxyphenyl acetic acid (DOPAC) were increased in the brain while no effect of restraint stress was observed on brain Dopamine (DA) level. Brain levels of 5-hydroxy indoleacetic acid (5-HIAA) was significantly decreased in restraint rats. Tryptophan levels in brain as well as in plasma significantly decreased in starved rats following 2hrs. restraint stress.

The findings are explained in terms of enhanced memory function following restrained stress which may be associated with a decrease in serotonergic function in starved rats.

INTRODUCTION

Stress is an important precipitant factor in depression and the changes in various body systems that occur in depression are similar to those observed in response to stress (Leonard, 2001). Previous studies have shown that behavioral changes occur after exposure to inescapable stress (Amat *et al.*, 2001). Episodes of 2hrs. restraint stress, produced marked anorexia in rats (Haleem and Perveen, 1994; Haleem *et al.*, 2002). The finding suggests ability of considering many life stresses that combine to effect depression and anxiety (Fisher *et al.*, 2001).

Deficiency of serotonin at important synaptic sites in the brain causes depression. This is evident by the observation regarding the alterations in 5-hydroxy tryptamine (5-HT) and 5-hydroxy indol acetic acid (5-HIAA) in patient during depressive episodes (Brown *et al.*, 1982). A decreased 5-HT and 5-HIAA concentration in cerebrospinal fluid has been reported in suicide victims and in aggressive individuals (Fisher *et al.*, 2001).

Repeated stress triggers a 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 (Adell *et al.*, 1988a,b). Adequate 5-HT system function supports regulation of intercommunicative neuronal transmission. It optimizes behavioral neuromodulation during and after different forms of exertions (Adell *et al.*, 1988a). A variety of stress stimuli increase the synthesis and catabolism of serotonin (Adell *et al.*, 1988a,b). It has been reported that different stress conditions such as restraint stress (Haleem and Perveen 1994; Haleem *et al.*, 1998) and

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starvation (Haleem and Haider, 1996) increase brain 5-HIAA levels. Behavior deficits also observed following restraint stress (Haleem and Perveen, 1994). Restraint and cold stress impair performance on memory test (Stillman *et al.*, 1998).

The present study was designed to monitor the effects of 2hrs. restraint stress on maze learning and on brain monoamine metabolism in starved rats.

MATERIALS AND METHODS

Animals and Treatment:

Locally bred male Albino Wistar rats of body weight 200-220g (purchased from PCSIR Laboratories, Pakistan) were used for the experiments. Rats were housed individually in plastic cages with sawdust covered floor in a quiet room with free access to cubes of standard rodent diet and water for 5 days before starting the experiment. Food intake and body weights were monitored daily. Growth rates were calculated as the percentage of initial day body weight (i.e. experimental day body weight/starting day body weight).

Before starting the experiment maze learning was also performed daily for about 5 days so that the animals may memorize or remember the whole maze. The width/size of the path in the maze was same everywhere. Animals were placed in the center of the maze for fixed interval of time, i.e., for 15 minutes. At one corner of the maze food was placed. The time in which animals reached to the goal was noted. The animals were starved for 2 days. Starvation was done by taking out diet from their cages and the next day the diet was again given for atleast 2hrs. After that maze learning was performed.

Following 2 days of starvation animals were restrained for 2hrs. Restraining procedure was same as described earlier (Haleem and Perveen, 1994). 24 hrs following restraint stress (Fig. 1),



Fig. 1: Restraint stress of rats on wiregrids.

animals were again placed in maze and memory was recorded. Animals were decapitated in alternate manner. Brain and plasma samples were collected immediately. Samples were stored at -40°C till analysis.

Neurochemical Analysis:

Concentration of brain 5-HIAA, NA, DOPAC and DA were estimated by High Performance Liquid Chromatography using EC detector.

Samples were extracted as described earlier (Haleem and Perveen, 1994).

A 4µm Novapak ODS, 4.6mm i.d. x 15cm separation column was used. The solvent system was methanol (14%), octyle sodium sulphate (0.023%) and EDTA (0.0035%) in 0.1 M phosphate buffer. Electrochemical detection was performed at an operating potential of 0.8V (glassy carbon electrode V/S Ag/AgCl reference electrode).

Statistical Analysis:

Behavioral and Neurochemical data were analyzed by Student's two tailed test and results are presented as means ± S.D. p values >0.05 were considered non-significant.

RESULTS

Fig. 2 shows effect of 2hrs. restraint stress on food intake and growth rate of starved rats. Data analyzed by Student's t-test exhibited significant effect of stress on food intake ($t=4.88$; $p<0.01$) and growth rate ($t = 6.89$; $p<0.01$). The unrestrained starved rats consumed an average of 6.07 gms of food while the restrained starved rats consumed an average of 2.167 gms of food. Thus 2hrs. restraint stress caused a reduction in food intake and in growth rate of starved rats which can be compared with unrestrained starved rats.

Fig. 3 shows the effect of 2hrs. restraint stress on maze learning in starved rats. Student's two tailed revealed a significant effect on maze learning ($t=4.84$; $p<0.01$). The results show that restrained starved rats take less time to reach the goal as compared to starved unrestrained starved rats.

Fig. 4 shows the effect of 2h restraint stress on brain levels of NA, DOPAC, DA and 5-HIAA. Data analyzed by t-test showed that brain levels of NA were significantly increased ($t=3.40$; $p<0.05$), brain levels of DOPAC were also significantly increased ($t = 3.14$; $p<0.05$) while there is a non significant effect on brain DA concentration in restrained starved rats.

Effect of 2hrs. restraint stress on brain levels of 5-HIAA showed a significant decrease ($t=3.74$; $p<0.01$) in 5-HIAA concentration of restrained starved rats. Levels of all the brain monoamines in restrained starved rats were comparable to unrestrained starved rats.

DISCUSSION

It was found that 2hrs restraint stress decreased 24 hrs. cumulative food intake and growth rate in rats (Haleem and Perveen, 1994; Haleem *et al.*, 1998). The present findings show that 2hrs. restraint stress significantly decreased food intake and growth rate in starved rats (Fig. 2).

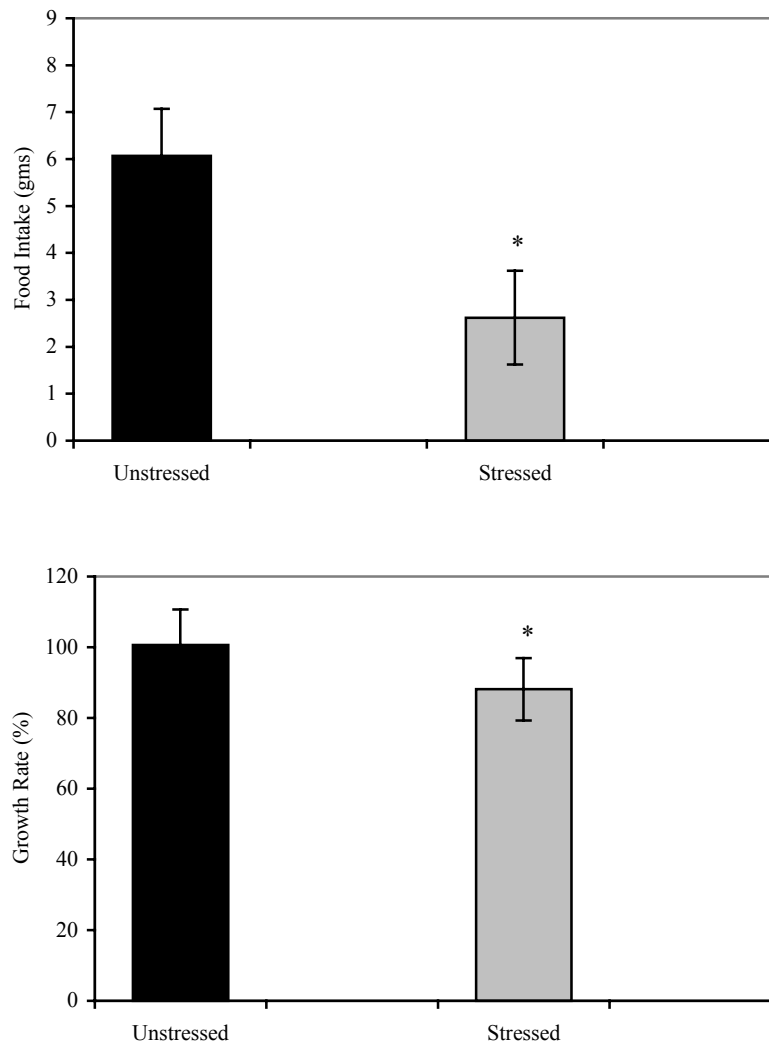


Fig. 2: Effects of 2hrs. restraint stress on food intake and growth rate in starved rats. Values are means \pm SD (n=6). Significant differences by students t-test * $p < 0.01$ from starved unrestrained rats.

An important finding of the present study is that the memory function is significantly increased (Fig. 3) in restrained starved rats. Previously it has been reported that memory is impaired in animals when they can no longer actively influence the outcome of a threatening situation (Siegfried *et al.*, 1990). Restraint and cold stress impair performance and memory task (Stillman *et al.*, 1998). The enhanced memory function in restrained rats observed in the present study (Fig. 3) could occur because the animals were starved and the goal was food.

Levels of brain NA and DOPAC are significantly increased in starved restrained rats (Fig. 4). It has been observed that acute experimental stress in rats increased the synthesis and release of NA and DA (Bills and Ailon, 1969, 1971). The results of present study show that 2hrs. restraint

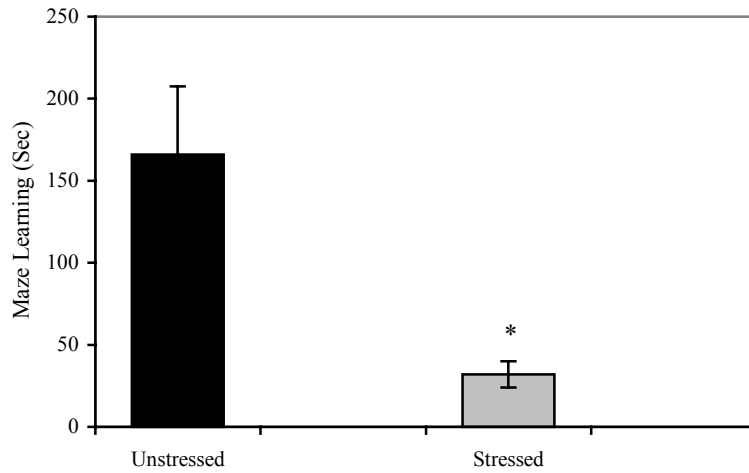


Fig. 3: Effect of 2hrs. restrain stress on maze learning in starved rats. Values are means + SD (n=5). Significant differences by students t-test *p<0.01 from starved unrestrained rats.

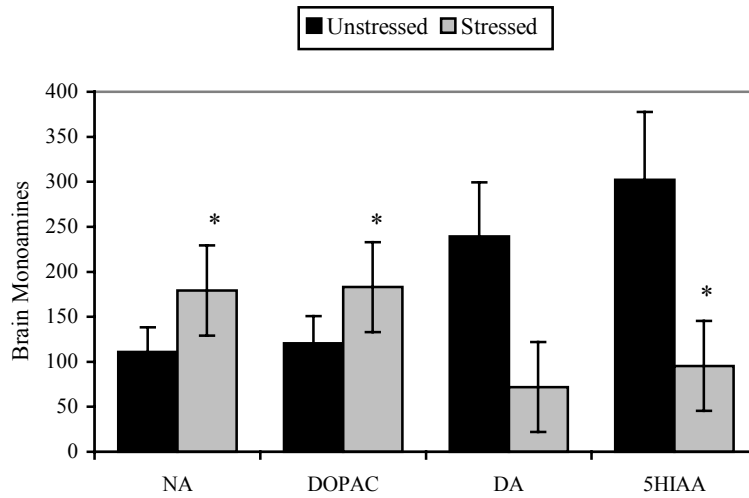


Fig. 4: Effects of 2hrs. restraint stress on brain monoamines in starved rats. Values are means + SD (n=5). Significant differences by students t-test *p<0.01 from starved unrestrained rats.

stress significantly decreased the levels of 5-HIAA in the whole brain of starved rats. It has been reported previously that the content of 5-HIAA, the main catabolite of 5-HT is increased in the brain of the rats exposed to acute experimental stress (Adell *et al.*, 1988; Dunn, 1984), foot shock stress (Malyszko *et al.*, 1994; Kennett *et al.*, 1986), restraint stress (Kennett *et al.*, 1986, Dunn and Welch, 1991) and starvation (Haleem and Haider, 1996).

The results of the present study show that 2hrs. restraint stress significantly decreased brain 5-HIAA levels in starved rats (Fig. 4). Decrease in brain levels of 5-HIAA of starved rats observed in present study may be due to acute stress and also may be due to starvation. Earlier, starvation related changes in HVA and 5-HIAA are observed in the hypothalamus (Tuomisto *et al.*, 1990).

Perhaps investigations involving more acute stressors along with prolonged starvation would produce more conclusive results.

CONCLUSION

Recent finding shows that restraint stress alters the functions of brain monoamines in starved rats. The most important and interesting finding of the present study is that 2hrs. restraint stress did not impair memory function in starved rats.

Effect of stress on learning and memory are complex. Under some conditions intense stress can produce pathologically strong and intrusive memories. Under other conditions, stress can impair memory to produce forgetfulness.

The present study therefore suggests that enhanced memory function following 2hrs. restraint stress is associated with a decrease in serotonergic function in starved rats.

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