RESEARCH ARTICLE

BEHAVIOURAL CHANGES IN ALBINO RATS DUE TO REPETITIVE HEAT STRESS OF MODERATE LEVEL

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ABSTRACT

Background: There are a number of studies showing the effect of severe heat stress on behaviour. Severe heat stress has been well reported to cause changes in activity level, attitude/mood, and behaviour. But, the effect of repetitive heat stress of moderate level on all these parameters has not been studied extensively.

Aims & Objective: In the present study, the effect of repetitive heat stress of moderate level was assessed on activity level, attitude / mood, and behaviour (spontaneous and provoked) of adult albino male rats (wistar strain).

Material and Methods: The experimental animals were subjected to repetitive heat stress for 4 hours daily, at 37±0.5°C in a Biological Oxygen Demand (BOD) incubator (relative humidity 65-82%) for five consecutive days. Daily changes were assessed in activity level during and after heat exposure and in attitude / mood along with behaviour (both spontaneous and provoked) after heat exposure in experimental animals and compared with control animals.

Results: There was increased restlessness during heat exposure and decreased level of activity after heat exposure in experimental animals. The mood of the experimental animals was depressed and the experimental animals kept them isolated and did not respond on provoking after heat stress.

Conclusion: All the above observations confirmed that exposure to repetitive heat stress, even of moderate level, causes significant change in behaviour of male albino rats.

KEY-WORDS: Provoked; Spontaneous Behaviour; Mood; Activity Level; Heat Exposure; Liver; Rat

Introduction

There are a number of studies showing the effect of severe heat stress on almost every organ in the body^[1-3] and on behaviour of exposed ones^[4-7]. It has been studied in detail and reported that severe heat stress caused changes in activity level, attitude, and behaviour. But, the studies related to the effect of repetitive heat stress of moderate level on structure and biochemical functions of various organs are few^[8,9] and one study is done by the same authors^[9]. In the similar way, there are very few studies^[10,11] in which the effect of repetitive heat stress of moderate level on behaviour has been observed. But, in these studies the effect on behaviour has not been studied extensively.

In the present study, unacclimatized adult albino male rats (wistar strain) were exposed to repeated heat stress of moderate level and observed for changes in activity level during and after heat exposure and in attitude / mood along with behaviour (spontaneous and provoked) after heat exposure.

Materials and Methods

Animals

12 Adult, male albino rats of Wistar strain, weighing 125–175 grams (5 -8 weeks of age) were used, following approval of Animal Ethical Committee of HIMS, Dehradun. The animals were housed in polypropylene cages (43cm X 29cm X 15cm) with a wire mesh top and a hygienic bed of rice husk under standard laboratory conditions with ad libitum access to water and freshly cooked food (a mixture gm/kg of porridge – 630, ground nut cake – 100, milk powder – 100, black gram – 100, salt - 10, and fish meal - 60). Each cage contained one to two animals. The room temperature was kept within thermo-neutral zone for albino rats at 25 ± 3°C (relative humidity 65 -85%), with the availability of normal day light. The experiment was conducted from September to December.

Experimental Protocol

The animals were divided into two groups control and experimental with 6 animals in each. All the experimental animals were exposed to moderately high environmental temperatures (37 \pm 0.5°C) in a BOD incubator (relative humidity 65-82%), 4 hours daily (11:00 am to 03:00 pm) for five consecutive days. Animals were kept fasting for 3-5 hours prior to heat exposure. During the heat exposure period, only one animal was housed in each cage with liberty of movement and with ad libitum supply of water but no food. Following heat exposure, the animals were restored to room temperature with ad libitum supply of food & water. Simultaneously, the control animals were kept at normal room temperature ($25 \pm 3^{\circ}$ C). Just like experimental animals, only one animal was housed in each cage and fasted for the same period of heat exposure with liberty of movement and with ad libitum supply of water.

Behavioural Observations

Daily changes in behaviour were noted and recorded, both in experimental as well as in control animals. For recording the behavioural changes, an observation checklist was adopted from the article, "Working with Rats in Research Settings"^[12] and the book, "Methods of Behaviour Analysis in Neuroscience"^[13] and modified according to the need of this work. In this checklist, differences between experimental and control animals were noted during and after heat exposure. During heat exposure changes were noted in activity level, while after heat exposure changes were noted in activity level. attitude/mood, spontaneous behaviour, and provoked behaviour. On the other hand, neurological changes, as tremor, convulsion, circling, paralysis, head tilt, and coma, were also sought to rule out any neurological damages, if any caused by moderate heat stress. The core body temperature (rectal temperature) of all the experimental animals was assessed before and after the heat stress to find out the core body temperature at thermoneutral zone $(T_{Re TN})$ and the post exposure core body temperature $(T_{Re HE})$ respectively. Along with these observations, to assess the heat stress, WBGT (Wet bulb globe temperature) index was calculated for animal room and for BOD incubator for five consecutive days of the heat exposure.[14-17]

Data Analysis

The data were analyzed by using both quantitative and qualitative techniques. Analysis of quantitative data was done by using the Student's unpaired (independent) t-test. All the data were expressed as means ± SEM. The program "Grap Pad instat" was used for these analysis.

Results

After single heat exposure at 37 \pm 0.5°C, T_{Re TN} raised from 34.88 \pm 0.257 °C to a statistically significant level (P value < 0.0001) of T_{Re HE} of 39.15 \pm 0.246 °C. Behavioural Observations are summarized in tables 1.

		Experimental (n=6)	Controls (n=6)
During Heat Stress	Activity Level	Restlessness	Normal activity
		Excessive running in the cage	No running in the cage
		Increased drinking of water	Normal drinking of water
		Soaked their body with saliva and urine	No soaked of their body with saliva and urine
After Heat Stress	Activity Level	Hypo activity	Normal activity
		Animals huddled, on placing them in cage	Animals not huddled
		Lethargic	Not lethargic
	Attitude/Mood	Depressed	Not depressed
		Disturbed sleep pattern	Normal sleep pattern
		Decreased activity	No decreased activity
		Not indulged in play	Indulged in play
		No weight loss	No weight loss
		No decreased food or water intake	No decreased food or water intake
	Spontaneous	Initially huddled but later on isolation	Neither huddled nor isolated from cage mates
	Behaviour*	from cage mates and not indulged in play	and indulged in play
	Provoked Behaviour	Minimal response	Normal response

Table-1: Effect of Moderate Heat Stress (4 hours/day for Five Consecutive Days at 37 ± 0.5°C) on Behaviour

* These observations were made without disturbing the animal.

There were no neurological changes, in experimental animals after heat stress. The calculated WBGT indexes for animal room and BOD incubator respectively were 24.4 \pm 0.30 and 36.5 \pm 0.03°C. This change in WBGT index was statistically significant (P value is < 0.0001). These results are similar to the results reported in another study done by the same authors on the effect of moderate heat exposure.^[9]

Discussion

The study of WBGT index clearly illustrated that on repetitively exposing animals even to moderately high temperature 37 ± 0.5 °C for 4 hours a day produce a significant heat stress to animals.

On the other hand, by this repetitive heat exposure of moderate level, there was statistically significant increase in $T_{Re\ HE}$ in comparison to T_{Re}_{TN} . It is evident that repetitive heat stress even of moderate level causes substantial hyperthermia though not of critical hyperthermia level.^[18]

The changes; observed in activity level during and after heat exposure, attitude/mood, spontaneous behaviour, provoked behaviour; and demonstrated that the animals exposed to repetitive heat stress of moderate level suffered from depression. These changes indicate that even though the heat stress is of moderate level, it may lead to significant changes in behaviour. The most probable explanation for these changes is that though the heat exposure is moderate but this lead to substantial hyperthermia of core body temperatures of experimental animals and the heat stress produced by this heat exposure was of statistically significant level as indicated by WBGT index. This hyperthermia and heat stress, most probably, produced psychological stress leading to above observed changes in behaviour.

Though the heat stress as observed by WBGT index was of statistically significant level but it failed to cause any neurological changes such as tremor, convulsion, circling, paralysis, head tilt, and coma. The most probable explanation for this may be that in core body temperature there was only substantial rise. If, the core body temperature have had raised to critical hyperthermia level, it has certainly produced the neurological changes.

While doing this study, few limitations were noticed but some of them were inadvertent such as small sample size which was the only size permitted by Animal Ethical Committee. Because of limited funds, the histological, morphometric, and biochemical study of brain was not done to support the behavioural and neurological findings. In future studies, it shall be tried to overcome these limitations.

Conclusion

All the above results demonstrated that when the male adult albino rats (wistar strain) were subjected to repetitive heat exposure even of moderate level, they suffered from heat stress of statistically significant level and substantial rise in core body temperature leading to changes in behaviour finally resulting in depression. While, no neurological changes were present in experimental animals indicating that moderate heat stress of short duration does not cause neurological damages.

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