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Heat acclimation alters the sleep and behavior based thermoregulatory dynamics of rats in heat stress

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Abstract

Background: It is well understood that the high environmental heat significantly affects the brain physiology of mammals, particularly the sleep and behavior of the subjects. The objective of the present work is to quantify the effects of acclimatization to the high environmental heat on sleep and behavioral activities in heat stress conditions.

Methods: The polygraphic data involved simultaneous recordings of cortical electroencephalogram (EEG), electrooculogram (EOG), and electromyogram (EMG) were recorded both on chart as well as in digital format to study the sleep-wake parameters in three different age groups of freely moving rats following exposure to high environmental heat. Each age group was subdivided into four groups: the acute heat stress group, subjected to a single exposure of 4h at 38°C in the biological oxygen demand (BOD) incubator; the chronic heat stress group, exposed for 21 days, for 1 h each day, at 38°C in the BOD; acute heat stress followed by 21 days of chronic heat acclimatization and the handling control group. Open field and elevated plus-maze behavior was also analyzed following different exposure setup of high environmental heat.

Results: The analyses of results suggest that acclimatization to the high environmental heat significantly alters the effects of acute exposure of high environmental heat on different sleep-wake as well as behavioral parameters.

Conclusion: Acclimatization to environmental heat shifts the thermoregulatory set-point and thus these altered changes in sleep and behavior have been observed.

Keywords: Acclimatization; Behavior; Heat stress; Sleep-wake parameters

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Background

The mechanism of thermoregulation in homeothermic animals is very complex. It involves behavioral as well as neuronal responses, which considers many levels of controls and interactions with different physiological processes [1]. However, the ultimate purpose of the thermoregulatory system is to preserve the stable temperature of the tissues and organs to maintain a constant core body temperature, while allowing the ambient temperature to vary. The hypothalamus, which is the chief center for regulation of body temperature, utilizes sensory information from external and internal affecters of the body for the thermoregulatory drive. Hypothalamus itself is responsible for further heat exchange with the environment through different physical and physiological mechanisms including sleep to keep the body metabolism in balance [2, 3].

Review of literature suggested that exposure to single acute heat induces the sleepiness with marked increase in slow wave

sleep (SWS) [4-7]. These studies indicate that increase in SWS is directly related with the conservation of energy in the thermoregulatory mechanism. Literatures also suggest that instantaneous increase in body temperature by exposing the subjects in acute environmental heat induces the synchronization of neuronal firing of the brain to compensate the heat by means of SWS [2, 3, 8-10].

Environmental conditioning in mammals is very difficult task and in varying environmental conditions, comparisons of sleep along with the hypothalamic activities are having great importance. It has been shown that heat acclimation or chronic heat exposure leads to elevation in body core temperature [11, 12]. The change in set point of body temperature indicates animal's behavioral and physiological adaptations in new environment, which ultimately enhance the thermal tolerance of the subjects. However, very few reports have been published to analyze the effects of heat acclimatization on the sleep and behavioral variables prior to acute exposure to high environmental heat. Libert et al. [13] have demonstrated the effects of chronic exposure of heat on sleep-wake stages with further recovery periods. Apart from this study, in such conditions when the subjects are primarily acclimatized to the similar environmental conditions for the adaptations in their physiological and behavioral activities, the effects on thermoregulatory mechanisms due to exposure to acute environmental heat is scantily studied.

Therefore, the objective of the present work is to quantify the effects of acclimatization on sleep and behavioral activities in heat stress conditions. The study has been planned to perform in a simulated model of environmental heat condition, where the animals have been acclimatized to the similar environmental conditions as subjecting them to acute heat stress.

Material and methods

Study design and subjects

Male Charles Foster rats of three different age groups (i) weaning – 4 to 5 weeks, (ii) young – 9 to 11 weeks and (iii) adult – above 6 months were used to carry out this experiment. Each group of rats was divided in three groups: (a) acute heat stress; (b) chronic heat stress and (c) acute heat stress to acclimatized subjects and further sub-grouped into stressed and control subjects. Similar number of subjects (n=5) in each group are taken for sleep and behavioral analysis. Each rat was separately housed in a polypropylene cages (30cm×20cm×15cm) with drinking water and food ad libitum and kept in artificially illuminated animal room with a 12 hours light cycle (light during 7.00 A.M. to 7.00 P.M.) at 24±1°C. The experiments were performed at laboratory of School of Biomedical Engineering, Banaras Hindu University, India.

Ethical committee approval

All procedures in this study have been passed by the Departmental Committee and conducted in compliance with the 'Committee for Purpose of Control and Supervision of Experiments on Animals', India as well as with internal Institutional policies and guidelines.

Electrode implantation

Electrodes for EEG (electroencephalogram), EOG (electrooculogram) and EMG (electromyogram) were implanted aseptically and chronically. The whole procedure of electrode implantation for recording of polygraphic sleep was conducted under Pentobarbital (35mg/kg i.p.) anesthesia as described by Sinha [14]. All animals, after implantation of electrodes, were allowed for seven days of post-operative recovery period and habituated with the recording environment before recording of the polygraphic signals.

Heat stress model

The stress was produced in the rats, by subjecting them in the Biological Oxygen Demand (BOD) incubator at preset temperature of 38±1°C and relative humidity of 45-50%, simulated with the environmental conditions of Varanasi (India) in the months of May and June [14].

Acute heat stress: Each rat of this group, following implantation of polygraphic electrodes and recovery from surgical stress was subjected for a single exposure in the incubator at the temperature of 38±1°C for four hours from 8.00 A.M. to 12.00 P.M. IST (Indian Standard Time), just before the recording of polygraphic signals.

Chronic heat stress: Rats were subjected in the BOD incubator for one hour daily for 21 days of chronic heat exposure from 8.00 A.M. to 9.00 A.M. at 38±1°C. The electrodes for polygraphic sleep recording were implanted on 14th day of chronic exposure to hot environmental heat, after the heat exposure. The recordings of electrophysiological signals were performed on 22nd day.

Acute heat stress to acclimatized subjects: After 21 days of chronic heat exposure, a separate group of rats were exposed to acute heat exposure on 22nd day before the recording of electrophysiological signals. The protocol for acute and chronic heat exposure was same as explained above.

Control: Rats were handled and processed as stressed groups of rats, respectively, but at controlled incubator temperature of

24±1°C (same as the room temperature). These groups of rats were treated as controls for their respective stressed groups.

Polygraphic recordings

Through 8 channel polygraph, continuous six hours sleep-wake recordings with help of EEG, EOG and EMG were performed from 12.00 P.M to 6.00 P.M. IST on the recording day for all rats at ambient room temperature of 24±1°C. The parameters of amplifier setting for different electrophysiological signals were set as described by Sarbadhikari et al. [15]. The digitized data, collected, stored and processed with the help of analog to digital converter (ADLiNK, 8112HG, NuDAQ, Taiwan) and its supporting data acquisition and processing software (VISUAL LAB-M, Version 2.0c, Blue Pearl laboratory, USA). The electrophysiological recordings were done with a sampling frequency of 256 Hz and selected data were stored at regular intervals in computer hard disk in two minutes segments in separate data files.

Behavioral activity monitoring

In the present study, the changes in behavior in rats were evaluated by Open-Field (OF) and Elevated Plus-Maze (EPM) methods. The open field experiments are mainly used to measure the fearfulness and reactiveness of the animals. However, the elevated plus maze is used to test the emotionality or anxiety of the subjects [16].

Open-Field: The field was a circular arena with the outer diameter being 84cm. There were 16 squares on the periphery. The inner concentric circle of 56cm diameter contained 8 squares. The 100W-frosted bulb was placed 1m above the field, an otherwise dark room during the activity testing. The behavioral parameters of each rat were tested in wake condition in OF for 3 minutes by placing the animal at the center of the apparatus are: (i) Immobilization: Rats had eyes open, holding its head against the gravity but without any head, body or limb movements. (ii) Grooming: Rhythmic paw movements over the face and/or head for face washing might include episodes of biting and cleaning of paws. (iii) Rearing: Standing still on upright on its hind limb only. (iv) Ambulation: When all the four limbs were in one particular square (central or peripheral) of the open field.

Elevated plus-maze

The maze had two open arms (50cm×10cm) and at right angle to it, two closed arms (50cm×10cm×40cm), with the roof uncovered; an open central crossing (10cm×10cm) and was rising to a height of 50 cm. The behavioral parameters of each rat were tested for 5 minutes in wake condition in EPM by placing them at the end of an open arm are: (i) Transfer Latency: Time taken (in seconds) by the animal to move from the outer end of the open arm to either of two closed arms. (ii) % Time in open arms: The percentage of total testing time spent in the open arm. (iii) % Time at central crossing: The percentage of total testing time spent at the crossing of open and crossed arms. (iv) Number of crossing of the arms: The number of times the animal crosses the center for going one arm to any other of the three arms.

Sleep analysis

Analyses of changes in sleep-wake time were done according to the method described by Andersen & Tufik [17]. Apart from the analyses of hourly changes in different sleep-wake states, data were also analyzed for (a) Total sleep time (TST): Sum of all sleep periods during the recording, (b) Total time of SWS (TSWS): Sum of all periods of SWS throughout the recording, (c) Total time of REM sleep (TREM): Sum of all periods of REM sleep throughout the recording, (d) Total wake time (TWT): Sum of all periods of waking during the recording, (e) Latency of SWS (LSWS): Time lag between onset of recording and the first episode of SWS and (f) Latency of REM sleep (LREM): Time lag between onset of recording and the first episode of REM.

Stress markers

Body temperature: The core body temperature of subjects was recorded through a six channel telethermometer. The probe was marked at 4 cm, inserted into rat's rectum and kept static for one minute to measure the body temperature. The body temperature was recorded on every third day just before putting the subjects into incubator for chronic stress groups. However, the recording of body temperature was done before and after the heat exposure in acute stress groups to monitor the change in body temperature. The recording of body temperature for control subjects was done in similar ways of their respective experimental (stressed) groups but the incubator temperature was kept at room temperature for control subjects.

Body weight

The changes in body weight of chronic stress group of subjects were examined on every third day of experiment by comparing the parameter with the respective control group of subjects. The body weight of acute group of subjects was recorded, before the surgical implantation of recording electrodes, three

days after the surgery, just before the stress and again just after the removal of rats from incubator.

These analysis of body weights of rats on different time schedule provide idea about the weight loss due to surgical stress in each group of animals.

Statistical analysis

All the statistical analyses were performed in the laboratory with the help of statistical software package (SPSS-7). The t-test was performed to compare data of different parameters following heat exposure with their respective controls.

Results

Assessment of heat stress

Changes in body temperature:

In the acute stress groups, the body temperature recorded after four hours of incubation were compared with body temperature recorded before the incubation at high temperature. The results showed that acute heat exposure significantly increased the body temperature in all three groups of rats such as in weaning ($P<0.01$), young ($P<0.01$) and adult ($P<0.01$), respectively. The analysis of results suggests that no alteration in body temperature was analyzed till 3rd day in all the three groups during chronic exposure to hot environment. It was found elevated in both weaning and young rats from 6th day onwards. But in adult group of rats, the rise in body temperature was analyzed only on 18th and 21st day (figure 1).

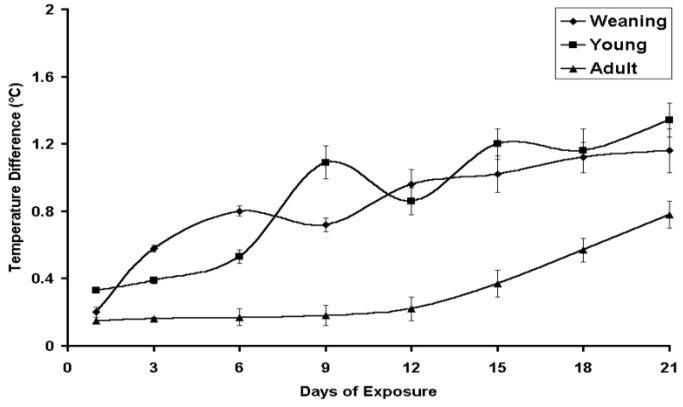


Fig - 1 :

Data expressed in mean±S.E. The differences in body temperature of (a) weaning group, (b) young group and (c) adult group of rats were recorded on 1st day and on every third day just before and after the rats were subjected to chronic heat stress.

Significant increase in the mean rectal temperature ($P<0.01$) of all three groups i.e. in weaning, young and adult rats on 21st day of chronic heat stress were analyzed. In comparison to the stressed subjects, heat acclimated rats have shown significant reduction in their mean core temperature as statistically significant reduction ($P<0.01$) in body temperature following acute heat exposure in acclimated rats have been shown in all three age groups of rats compared to their respective unacclimated acute groups (figure 2).

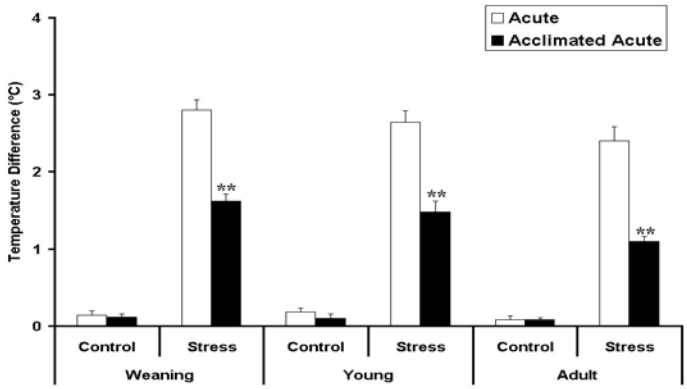


Fig - 2 : Data expressed in mean±S.E. The body temperature of all three age groups of animals were recorded before and after the acute heat exposure compared to control groups, **$P<0.01$.

Changes in body weight:

The statistical analyses show that there were insignificant changes in the body weights in any group of animals following surgical stress of electrode implantation and followed by acute heat exposure. In weaning and young groups of rats, reduction in body weights were observed due to chronic exposure to hot environment, while the adult rats did not show any significant change in body weight as compared to the control group. Observations from weaning group show significant decrease in body weight from 6th day onwards ($P<0.05$ or better), while on 21st day, the decrease in body weight was observed maximum ($P<0.01$). On the other hand, the young rats show significant change in body weight only on 18th and 21st day ($P<0.05$) of the chronic stress (figure 3).

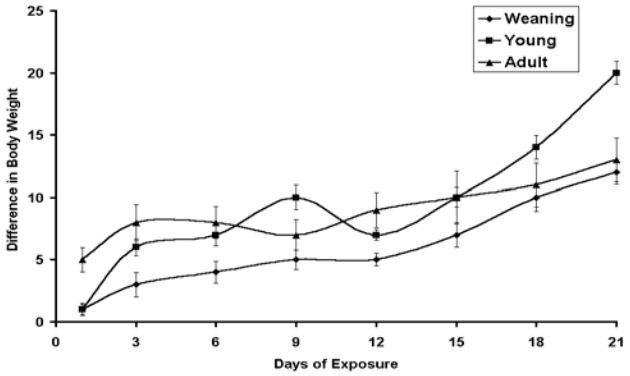


Fig - 3 : Data given in mean±S.E. The change in body weights

were recorded in (a) weaning, (b) young and (c) adult group of rats on 1st day and on every third day of chronic heat stress.

Evaluation of changes in sleep parameters

The analyzed results for alterations in sleep-wake parameters in three different experimental conditions are summarized in table 1. Following acute heat exposure, in young and adult, TST and TSWS were significantly increased (P<0.05), while significantly high increase in TSWS (P<0.01) was analyzed in weaning group. The weaning rats also showed significantly decreased TREM (P<0.05), however, other two groups of rats show suppressive but insignificant changes. In young and adult groups of rats, TWT was significantly reduced (P<0.05) following acute heat stress. The LREM was significantly increased in all age groups of animals (P<0.05) and the LSWS was significantly decreased in weaning and increased in young groups of rats (P<0.05) following acute heat stress.

Table 1: Comparative effects of acute, chronic and acclimatization to heat stress on sleep-wake parameters (mean±S.E.)

Weaning				
	Control	Acute	Chronic	Acute after heat acclimation
TST	252.8±9.60	243.6±8.54	172.6±8.94†	217.0±10.22†
TSWS	156.6 ±7.79	182.2 ±7.04†	116.8±5.32†	152.8±6.62
TREM	96.2±6.23	61.4±5.85*	55.8±6.28†	64.2±5.42*
TWT	107.2±9.27	116.2 ±7.62	187.4±9.52†	143.0±8.81†
LSWS	32.0±3.67	27.2±3.61*	21.2±4.43*	24.6 ±3.87*
LREM	39.4±6.97	46.4±6.22*	52.0±5.33*	45.4±7.42
Young				
	Control	Acute	Chronic	Acute after heat acclimation
TST	226.8±8.10	250.2±8.54*	203.8±9.73*	225.2±9.38
TSWS	142.2±6.46	159.2±5.17*	128.4±6.28*	135.0±8.20
TREM	84.6±7.68	91.0±6.5	75.4±7.88	90.2±8.76
TWT	143.2 (8.49	110.0±8.65*	146.2±9.33	134.8±9.37
LSWS	24.0±5.33	38.6±5.44*	15.4±3.43*	26.6±4.28
LREM	36.8±6.48	46.4±4.97*	29.8±4.76*	32.8±4.61
Adult				
	Control	Acute	Chronic	Acute after heat acclimation
TST	218.2±10.65	247.0±8.62*	235.6±8.97*	205.0±7.78*
TSWS	181.4±6.93	204.0±8.61*	191.8±7.24	156.6±5.88*
TREM	36.8±3.57	43.0±4.22	43.8±4.85	48.4±3.97
TWT	141.8±9.67	113.0±7.38*	124.4±6.55*	155.0±6.59
LSWS	20.2±4.2	26.8±3.47	28.0±5.36*	31.4±4.16
LREM	39.2±5.35	52.6±5.24*	50.4±5.88*	34.6±3.83

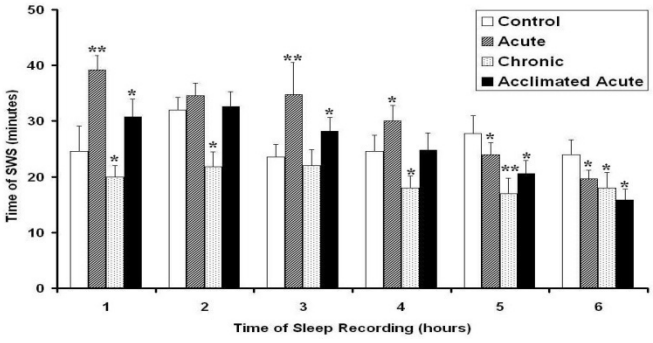
†P<0.01, statistically significant
*P<0.05, statistically significant

Compared to increased TST and insignificant changes in TSWS as analyzed in adult rats, following 21 days of chronic heat exposure, in weaning and young groups of rats, the TST and TSWS were observed significantly decreased (P<0.05). Adult group of rats showed significant decrease in TST (P<0.05) only. However, the TREM was significantly decreased only in weaning rats (P<0.01) and found unchanged in other two (young and adult) groups. The statistical analyses of the data of waking episodes showed a significant increase in TWT in weaning (P<0.01) and adult (P<0.05) groups. While no statistical changes were analyzed in young subjects. The results also showed fragmented sleep as significantly large number of sleep stage changes was counted in these groups. The LSWS was significantly decreased in weaning and young groups (P<0.05) and significantly increased in adult rats (P<0.05). The LREM was observed significantly increased in weaning and adult groups (P<0.05) and significantly decreased in young group of animals (P<0.05).

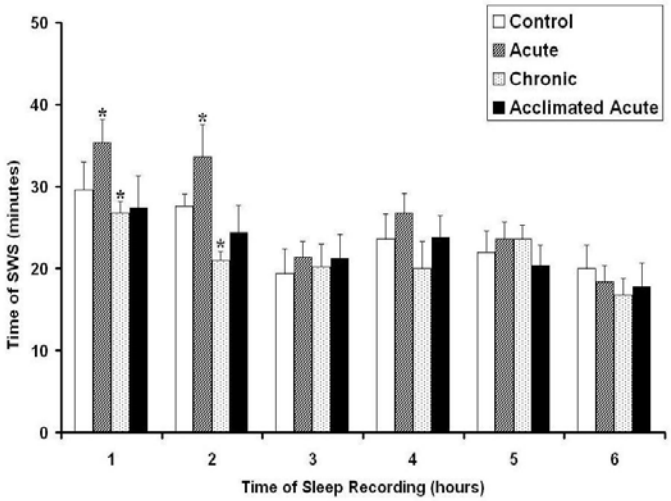
Many changes appeared due to acute heat exposure in different experimental groups of rats were analyzed to be reversed after chronic acclimatization of heat stress. In acclimatized groups of subjects, following acute heat exposure, significantly reduced TST was analyzed for weaning and adult groups of subjects (P<0.05 or better) in comparison to their respective control groups. Significant reduction in TSWS in adult subjects was also monitored (P<0.05). The above alterations in sleep-wake parameters were analyzed to be significantly different from acute exposure groups. Apart from changes in TST, weaning group of subjects were also shown significantly increased TWT, reduced TREM and LSWS (P<0.05 or better).

The statistical analyses of results also revealed that acclimatization significantly reduces the TSWS in each hour of sleep-wake recording, when compared to the acute heat exposure groups of subjects. Similar results have been examined in all three age group of rats (figure 4a-c).

(a) Weaning group



(b) Young group



(c) Adult group

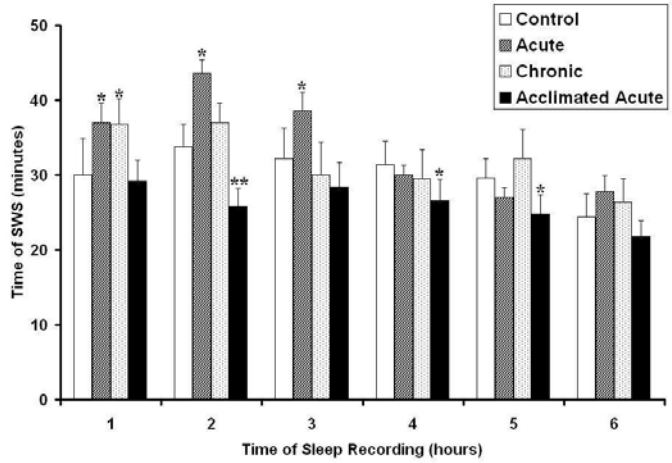
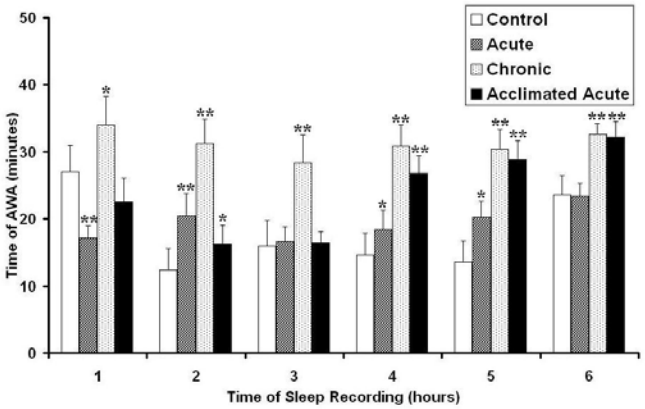


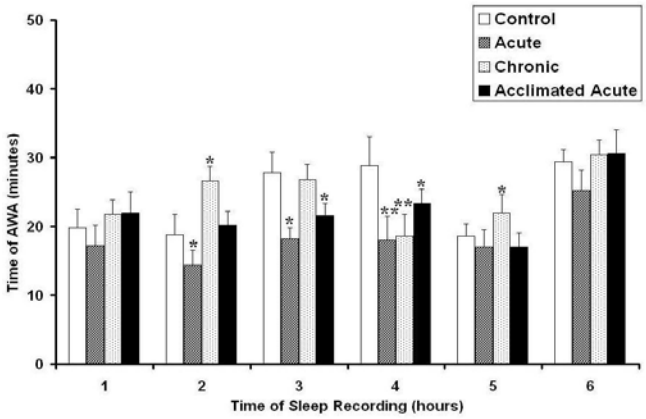
Fig- 4 show the comparative study (mean±S.E) of the effects of acute, chronic and acclimatization to heat stress and compared (**P<0.01, *P<0.05) with the control subjects for (a) weaning, (b) young and (c) adult groups under slow wave sleep (SWS).

In general, reverse results have been examined in hourly recording of AWA time (figure 5a-c).

(a) Weaning group



(b) Young group



(c) Adult group

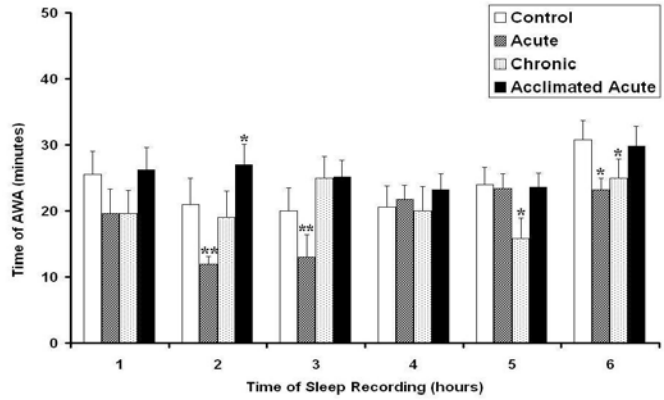
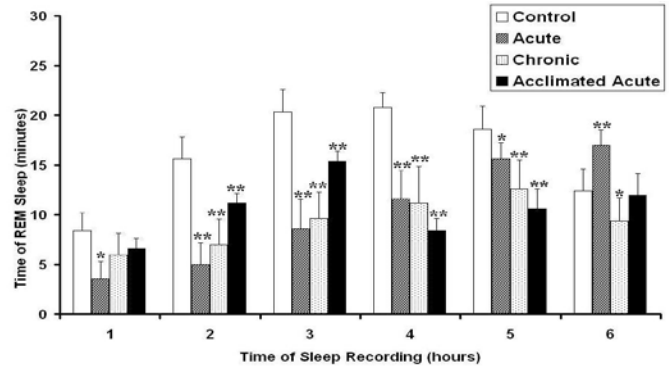
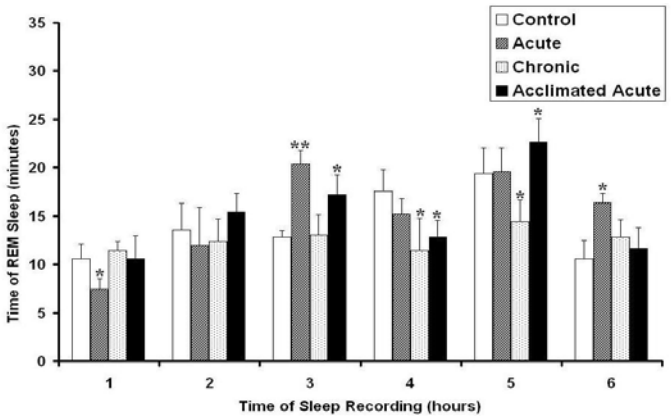


Fig- 5: Figures show the comparative study (mean±S.E) of the effects of acute, chronic and acclimatization to heat stress and compared (**P<0.01, *P<0.05) with the control subjects for (a) weaning, (b) young and (c) adult groups under awake state (AWA). However, the REM sleep in hourly recording does not showed much difference in average, when compared with the single exposure acute heat stress groups of subjects. While one interesting phenomena has been noted in all groups of subjects. The REM sleep was monitored to be showing increasing trend for first two hours in young and adult groups of subjects and for first three hours in weaning group, when compared with single exposure acute stress groups (figure 6a-c).

(a) Weaning group



(b) Young group



(c) Adult group

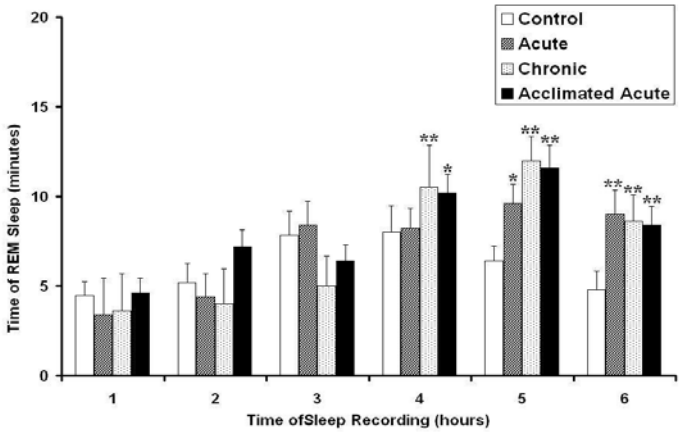


Fig-6. Figures show the comparative study (mean±S.E) of the effects of acute, chronic and acclimatization to heat stress and compared (**P<0.01, *P<0.05) with the control subjects for (a) weaning, (b) young and (c) adult groups under rapid eye movement (REM) sleep.

In OF, highly significant increase in immobilization (P<0.01) accompanied with decrease in rearing (P<0.05) was observed in all age groups of experimental subjects, while decreased grooming (P<0.05) was also observed in young and adult subjects.

Evaluation of changes in behavioral parameters

The data of behavioral analyses following acute heat stress with respect to the control groups (table 2a-c), show significant changes in animal behavior in OF and EPM.

All the three age groups of rats showed similar trend in decrease in ambulation behavior (P<0.05) such as in peripheral squares, central squares and total squares as observed following acute exposure to high heat. On the other hand, in EPM, significant decrease in time spent in open arm and number of arms crossed

Table 2. The effects of heat stress on behavioral parameters on open field and elevated plus maze are compared (mean±S.E.)

	Control	Acute	Chronic	Acclimated Acute
Weaning				
Open Field				
Immobilization	17.6 ±3.80	98.8±6.78 †	26.0±2.13	42.8 ±4.67 †
Rearing	14.2±2.33	8.2±2.45*	12.0 ±1.97	10.2 ±2.31*
Grooming	7.8±1.52	6.4±1.32	10.4 ±0.98	7.6 ±1.48
Ambulation (Squares)				
Peripheral	62.2±3.21	40.6±5.25*	74.8 ±5.87	55.4 ±2.38*
Central	10.6±2.11	7.0±1.50*	7.6 ±2.43	6.8 ±1.97
Total	72.2±4.82	47.6±3.68*	82.4±6.63	62.2±7.85
Elevated Plus Maze				
Transfer latency	24.6 ±2.87	38.8±2.25*	21.8 ±3.74	26.4 ±2.69
% Time (Open arm)	20.35±1.78	7.5±1.56†	24.56 ±3.25	14.33 ±2.46*
% Time (Center)	5.85 ±1.23	4.33±1.11	7.62 v1.64	5.56 ±1.05
No. Arms crossed	6.4±0.87	3.4±0.54*	10.2 ±0.72*	7.2±1.00
Young				
Open Field				
Immobilization	21.2±2.79	104.4±5.35†	24.8 ±2.21	60.4 ±4.38†
Rearing	7.0±0.89	3.4±0.92*	8.8 ±0.77	6.6 ±1.24
Grooming	9.4±0.92	5.4±0.60*	5.6 ±0.78	5.2 ±0.79*
Ambulation (Squares)				
Peripheral	58.2±3.74	47.4 ±1.64*	61.8 ±2.70	52.0 ±4.69
Central	8.8±1.03	3.4 ±0.67*	8.4 ±1.08	5.4 ±0.53
Total	67.0 ±4.38	50.8 ±2.10*	70.2 ±3.13	57.4 ±5.83
Elevated Plus Maze				
Transfer latency	22.4±2.24	23.8 ±1.10	20.0 ±2.84	22.8 ±2.78
% Time (Open arm)	17.8 ±1.58	9.83 ±1.23*	18.4 ±2.27	12.67 ±1.64*
% Time (Center)	7.4 ±1.08	5.0 ±0.09*	8.4 ±1.59	6.33 ±1.19
No. Arms crossed	7.8±0.77	3.8 ±0.59*	9.2 ±0.59*	5.2 ±1.16
Adult				
Open Field				
Immobilization	24.8 ±4.21	112.8 ±8.42 †	22.6 ±3.74	64.6 ±6.73 †
Rearing	6.4 ±1.74	3.4 ±0.92*	6.2 ±1.34	4.2 ±1.02
Grooming	8.6 ±0.79	4.8 ±1.04*	5.4 ±1.27	5.0 ±1.34
Ambulation (Squares)				
Peripheral	60.4 ±6.34	40.6 ±4.35*	63.6 ±5.28	54.2 ±5.87
Central	9.2 ±2.31	5.4 ±1.42*	8.2 ±1.36	7.4 ±1.90
Total	69.6 ±7.32	46.0 ±4.86*	71.8 ±6.73	61.6 ±5.64
Elevated Plus Maze				
Transfer latency	16.6 ±3.17	25.0 ±2.67*	21.4 ±2.91	24.2 ±2.22*
% Time (Open arm)	14.1 ±2.52	9.17 ±1.49*	17.8 ±2.21	11.5 ±2.56
% Time (Center)	8.7 ±1.67	5.78 ±1.14*	7.8 ±1.46	6.75 ±1.57*
No. Arms crossed	11.2 ±1.21	3.2 ±1.02 †	9.4 ±1.63	8.4 ±0.85

† P<0.01, statistically significant
* P<0.05, statistically significant

(P<0.05 or above) were analyzed in all age groups of experimental subjects. While significant increase in transfer latency (P<0.05) in weaning and adult subjects and decrease in percentage time spent on center (P<0.05) in young and adult groups of subjects were also recorded.

Except significant increase in number of arms crossed in weaning and young groups of subjects (P<0.05), following 21 days of chronic exposure of high environmental heat, changes in behavior in OF and EPM were analyzed to be insignificant. In 21 days, acclimatized subjects of all age groups, followed by acute heat exposure had shown minimized effects of hot environmental stress on their behavior. Many of the recorded alterations in these groups of rats were found to be significantly reduced in comparison to the single acute heat stress group of subjects; such as immobilization in all age groups, peripheral ambulation in weaning group, percent time in open arm in weaning and young subjects and percent time in center in adult group of rats.

Discussion

The primary response to the heat is the immediate rise in body temperature that play the central role in stimulating the mechanism necessary to heat dissipation, vasodilation and sweating. Animals, when subjected to hot environment, responded by activating different physiological processes. The intensity, duration and the adaptations to hot environment play important role in change of many physiological processes and determine the level of thermoregulatory activity, which influences the performance of all animals including man. These adaptational adjustments can also give way to more prolonged metabolic changes associated with growth and reproduction during life in hot climate [11].

Analysis of Changes in Sleep-Wake cycle

The results of the present study showed that acute heat stress significantly increased the TSWS and LREM in all three age groups of rats. Consequently, TWT was analyzes to be decreased in young and adult groups. However, it is interesting to note that the weaning subjects did not showed such significant decrease in TWT, while they showed significant decrease in TREM. On the other side, following 21 days of chronic exposure of hot environment, all three groups of rats showed statistically different behavior as weaning and young groups showed decrease but increase in adult group for TST and LSWS. Following 21 days of adaptations and then subjected to the acute heat stress, rats showed some interesting outcomes. In this group of experimental subjects, the changes appeared due to either acute or chronic heat stress were nullified as majority of parameters showed statistically insignificant changes as analyzed in young and adult groups. The weaning subjects generally not followed the path of young and adult subjects and showed some interesting variation in four different sleep parameters, in which three of them were reflective as they have already displayed in chronic heat stress.

The increase in TSWS was also reported in different age groups of rats under acute exposure to high environmental heat. The TWT was found inhibited, which is similar to the observations as reported earlier [2, 3]. It has been earlier suggested that increase in body temperature both by passive heating of ambient heat or by exercise, leads to increased SWS and theorized that increase in SWS after transient increase in body temperature represent an active thermoregulatory response triggered to counter hyperthermia [18]. Similar to the previous findings, the results of the present study also indicated that TST is a function of thermoregulatory drive as hypothalamic temperature has been shown to have a profound effect on sleep in a variety of mammalian species and it has been suggested that TST was closely correlated with the thermoregulatory error signal defined as the difference between the hypothalamic temperature threshold for the heat production response and actual hypothalamic temperature [19].

It has also been reported that acute heat stress at 38°C induces significant increase in serotonin level in rats [20]. A significant correlation between increase in SWS and increased concentration of serotonin has already been demonstrated [21, 22]. Thus, the increased SWS in rats subjected to acute heat stress as observed in this study might also be due to quantitative alterations in serotonin level, which may also be influenced by several other neurochemicals in brain. The present investigation indicates that total time spent by the weaning rats in REM sleep decreased following acute exposure to 38°C. The latency of appearance of REM was also noted to be significantly increased in all the three age groups of rats. This is in accordance with the data of recent research, which suggests that serotonin inhibits cholinergic laterodorsal tegmental (LDT) activity and this in effect suppresses REM activity [23-25]. Because rat LDT has greater serotonin innervations, the observations suggest that serotonin may act the LDT to inhibit REM sleep in low age group of rats, supports our findings in decreased REM sleep in weaning group of rats following acute exposure to high environmental heat [23]. In these rats, lyposaccharides elicited a longer suppression of REM sleep. The delayed appearances of REM sleep, found in the present study

may be correlated with the increased lyposaccharide concentration in rat brain due to hyperthermia induced by acute exposure to high environmental heat as suggested earlier [26]. Furthermore, this study also shows a decreased latency of SWS in weaning group and increase in young group of rats. As this state generally constitutes the transition from AWA state to SWS, these variations indicate the age related changes in thermoregulation following acute heat stress, and may have direct consequence with these results. It has been shown that aging is strongly accomplished by changes in hypothalamo-pituitary axis (HPA) function, which is directly related with the thermoregulation in hot environment [26-28].

Apart from involvements in feeding activities, the perifrontal lateral hypothalamic area (LHA) has also been implicated in sleep-wake control and locomotor activities [29-31]. Alam et al. [29] have hypothesized that hypocretin neurons of LHA exhibit a wake related discharge patterns, with peak discharge in waking state. Thus, activation of hypocretin neurons of LHA following chronic exposure to hot environment may be responsible for the increase in AWA, with corresponding decrease in SWS, and finally result in decrease in TST in weaning and young rats. Young and adult rats behave differently in their sleep promotion following chronic heat stress as LSWS and LREM were observed delayed in adult rats, while early promotion of sleep (decreased LSWS and LREM) were observed in young rats. However, the weaning group of rats show only increased LREM with decreased LSWS. These reverse effects of the chronic hot environment on these experimental rats have not been well understood and supposed to have occurred due to differential thermoregulatory effects on hypothalamus, which may be evident in the results of changes in body weight and body temperature in these rats following chronic heat stress.

The review of literatures suggest a complex brain function following chronic heat stress that involves various neuronal and non-neuronal systems in alterations in sleep-wake states in all three groups of rats in the present study, which finally disrupt the sleep-wake behavior in these rats as number of sleep stage changes were significantly increased in all the three experimental age groups following chronic heat stress [30]. The change in set point of body temperature following chronic heat stress, and disturbed thermoregulatory systems of hypothalamus might be the main factor responsible for the upset of sleep-wake behavior in the rats of all age groups.

The changed sleep-wake activation in weaning group compared to young and adult group of rats, following acute and chronic heat stress may have occurred due to immature membrane properties of neuronal circuit [32]. It has been suggested that in contrast to adult neurons, whose energy is provided almost entirely by glucose, the weaning brain obtain most part of its energy from ketone bodies and other products of the lipid metabolism [33, 34]. Due to these factors, the oscillations within the intrathalamic and thalamocortical circuits are found changed in weaning rats in comparison to young and adult rats, and thus produce age dependent changes in EEG and sleep-wake activities following acute as well as chronic heat stress [35].

Analysis of behavioral changes

Results of the present study showed that with prolonged single exposure of high environmental heat induced increase in immobilization in OF and transfer latency in EPM. Along with these changes, decrease in rearing, grooming and ambulation also revealed that the acute heat exposure caused fatigue and altered excitability of nervous system. Later on, in EPM, animals prefer to stay in the closed arm of the apparatus that may be due to the search of protective shelter and eventually they were found to fall in the state of drowsiness, resulted in decrease in the number of arms crossed and percent time spent on open arms. While on other hand, chronic exposure to heat stress, leads toward the adaptation of subjects to the stressful environment. They showed rare behavioural changes on 21st days of behavioural examination. It is very interesting to know that acute exposure to environmental heat following 21 day of heat acclimatization results in comparatively less alterations in behaviour in animals. The main alteration that still persists was the significant increase in immobilization in OF in all age group of experimental subjects, which may have occurred due to fatigue owing to sever heat. The acclimatized followed by acute heat stress group shows almost similar pattern of behaviour in EPM.

The body temperature of the animals is maintained within fairly narrow limit by thermoregulatory mechanism that rely on large number of graded physiological and behavioural responses depending on the thermal nature of the environment [8]. It has also been well established that biochemical and cellular mechanisms are highly temperature sensitive [36-39]. Elevation in body temperature due to heat stress is known to stimulate the limbic areas of brain and pathways projecting from these areas to the hypothalamus, stimulates corticotrophin releasing hormone (CRH) secretion into HPA axis. It also stimulates various other neural pathways in the brain [40-42]. Beside, CRH not only stimulates pituitary to increase the corticotrophin

secretion, but also increase the sympathetic outflow to the adrenal resulting in increased output of both cortical and modularly hormones that may cause behavioural abnormalities in the subject [43].

Whether or not set point temperature has changed when body temperature changes in response to an environmental challenge has been focused in many studies [44-46]. The set point theory is based on the principle that the thermoregulatory system under environmental adaptations operates continuously to maintain the core temperature and there is a balance between activation of heat gain and heat loss thermoeffectors. A change in set point temperature under heat stress leads to marked changes in the thermoregulatory response. Physiologists have suggested a narrow range of regulated temperature that represent that the composite set point of several thermosensitive areas and several different thermoregulatory responses [8] and the body set point temperature is not a static one rather is flexible and peripheral input can alter it. Some preoptic neurons not only sense local temperature but also receive synaptic input from afferent pathways of thermoceptors in skin, spinal cord and other locations throughout the body [47-50]. The neurons of preoptic area integrate central and peripheral thermal signals and control a particular temperature by shifting this preoptic set point temperature [8].

Study limitations and future scope

This study was performed to analyze the effects of heat stress on sleep-wake cycle and behavioral parameters of the rats. The correlations of the changes in each parameter with different biochemical levels are not been evaluated. The evaluation of correlation between different biochemicals with the alterations in sleep-wake patterns as well as behavioral parameters will definitely help in study the effects of acute and chronic heat stress on central nervous system.

The explanation of dynamic set point temperature and adaptations of thermoregulatory responses under chronic heat exposure supports the explanation of our results in this study. Further, based on the analysis of results of this study and the review of literature, it is suggested that the thermal adaptations due to chronic exposure to the high environmental heat produces long term adaptational changes, which lead to significant reduction in the acute effects of high heat on sleep and behavior of the subjects.

Conclusion

Both acute as well as the chronic exposure affect the thermoregulatory pathway of the mammals. Although, under high environmental heat, exact neural as well as biochemical mechanism is still not completely explored, it is sure that the exposure to heat stress alters the physiology of HPA and due to this changes in sleep-wake patterns and behavioral activities are evident. Further, chronic exposure to hot environment leads to the adaptational adjustments in many physiological responses including sleep and behavior of the subjects.

Abbreviations

Biological oxygen demand (BOD), electroencephalogram (EEG), electromyogram (EMG), electrooculogram (EOG), elevated Plus-Maze (EPM), latency of REM sleep (LREM), latency of SWS (LSWS), open-Field (OF), slow wave sleep (SWS), total sleep time (TST), total sleep time (TST), total time of REM sleep (TREM), total time of SWS (TSWS), total wake time (TWT).

Competing interests

Authors do not have any competing interests.

Authors' contribution

Sinha RK designed the study, performed the experiment, interpreted the data, analyzed, drafted the manuscript, and revised it.

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