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SLEEP CHANGES DURING CHRONIC COLD EXPOSURE SHOWED THAT THE HOMEOSTATIC REQUIREMENT OF SLEEP IS REDUCED IN THE MEDIAL PREOPTIC AREA LESIONED RATS

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Abstract : The effects of chronic exposure to a mildly cold ambient temperature (T_a) of 18°C on sleep wakefulness (S-W) and brain temperature (T_{br}) were studied in the medial preoptic area (mPOA) lesioned male Wistar rats. Electroencephalogram (EEG), electrooculogram (EOG) and electromyogram (EMG) electrodes were chronically implanted to assess S-W, and a thermocouple above the dura to record the T_{br}. After three recordings (24 h each) of S-W and T_{br} at 24°C, N-methyl D-aspartic acid (NMDA) was intracerebrally injected to produce bilateral destruction of neurons in the mPOA. There was decreased sleep and increased T_{br} even four weeks after the mPOA lesion. T_a of the environmental chamber was then reduced to 18°C, and the S-W and T_{br} were again recorded for 24 h each on the 1^{st}, 7^{th}, 14^{th}, 21^{st}, and on 28^{th} days of continuous exposure to the mild cold T_a. Exposure to the cold produced further decrease in sleep and increase in the T_{br}. However, sleep came back to the pre-exposure level by the 14^{th} day. An increase in the duration of sleep episodes was responsible for the restoration of sleep during chronic cold exposure.

The study showed that the requirement of sleep was reset at a lower level in the mPOA lesioned rats. The mPOA lesion affected the sleep maintenance and sleep initiation, though the latter became evident only during chronic cold exposure. The magnitude of the acute changes in T_{br} and S-W were less in the lesioned rats, as compared to those observed in the normal rats exposed to similar cold T_a. On the basis of these observations, it could be proposed that the mPOA plays some role in cold induced changes in thermoregulation and sleep regulation. The T_{br} remained elevated throughout the period of cold exposure. Resetting of the T_{br} at a higher level may be part of the homeostatic readjustment to restore sleep.

Key words : ambient temperature chronic cold exposure sleep mPOA lesion brain temperature

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INTRODUCTION

Acute exposure of rats to mild cold ambient temperature (T_a) of 18°C produced a reduction in sleep (1–4). But, there was homeostatic restoration of sleep during chronic exposure to mild cold T_a (4). There was also an increase in brain temperature (T_{br}) on exposure to cold T_a. It was suggested that the increase in T_{br} contributed towards homeostatic restoration of sleep as it has been experimentally shown that the warming brain can promote sleep (5–8). The medial preoptic area (mPOA) of the basal fore brain is one area which is implicated not only in the regulation of sleep and body temperature, but also the site from where sleep can elicited by local brain warming (5–7, 9). This area also plays a critical role in interlinking thermoregulation and sleep regulation (9–18). This investigation was undertaken to find out the role of the mPOA in the restoration of sleep during chronic cold exposure.

Earlier electrolytic lesion studies had shown that the physiological thermoregulatory mechanism is grossly affected after the preoptic lesion (14, 20). But, subsequent neurotoxic lesion studies, which preferentially destroyed the neurons, leaving the fibres by and large intact, showed that the physiological thermoregulation was unaffected after the destruction of the mPOA neurons (17, 21). But, the body temperature and T_{br} were elevated after the mPOA neuronal destruction (18, 21). The increase in body temperature after the mPOA lesion was described as a resetting of thermostat, rather than a failure of thermoregulation (16, 17, 21). When the mPOA lesioned rats were given freedom to select their own T_a, they moved over to a high T_a. This action could be a behavioural thermoregulation aimed at increasing the body temperature and T_{br}. Though sleep was reduced after the mPOA lesion, it was considerably restored when the rats voluntarily selected a higher T_a. Thus, it is possible that this increase in body temperature, which is described as a thermostat resetting at a higher level, may be aimed at homeostatic restoration of sleep. Study of the changes in T_{br} and S-W in the mPOA lesioned rats, when chronically exposed to mild cold T_a, would provide information about the role of this area in interlinking thermoregulation and sleep regulation.

An increase in the number of sleep episodes was responsible for the partial restoration of sleep in the mPOA lesioned rats (21). During chronic cold exposure also, sleep was restored by an increase in the number of sleep episodes (4). This suggests the possibility that sleep could be restored by increasing the number of sleep episodes during chronic cold exposure, even after the destruction of the mPOA neurons. But, this probability needs to be substantiated by experimental evidence. So, a study of S-W and T_{br} in the mPOA lesioned rats would provide information, not only about the role of the mPOA in the restoration of sleep during chronic cold exposure, but also about the homeostatic process that regulate sleep.

MATERIAL AND METHODS

The experiments were conducted on male Wistar rats weighing 185–285 g obtained from the Experimental Animal Facility of

the All India Institute of Medical Sciences, New Delhi, India. They were kept in an animal room with the temperature maintained at 24 ± 1°C with 14 h light (illumination above 200 lux) and 10 h dark (illumination below 5 lux) schedule. Under sodium pentobarbital anaesthesia (40 mg/kg, bw. ip), these animals were implanted with electrodes for recording EEG, EMG and EOG to assess S-W. A thermocouple was placed on the right parietal cortex above the dura (2 mm posterior to bregma and 2 mm lateral to mid sagittal suture) to record the T_{br}. Two tiny holes were drilled 0.6 mm lateral to the mid sagittal suture and 2 mm anterior to the bregma, for the microinjection of N-methyl D-aspartic acid (NMDA) (Sigma Chemicals Co., USA) to lesion the mPOA. Seven days after the operation, when the rats had completely recovered from the operative trauma, they were placed in a sound proof environmental chamber, wherein the temperature and light-dark schedule were kept identical to those of the animal house. The leads were taken through a micro-swivel to avoid the entangling of wires during the movement of the animals. The IC socket was connected with leads for recording the EEG, EMG, and EOG in the computer using Biopac software (Biopac Systems, Inc. USA). At every 30 s interval, T_{br} was recorded by an electronic digital thermometer (Fluke, USA), connected to a computer.

Recordings of EEG, EMG, EOG and T_{br} were taken for 24 h on three alternate days when the rats were maintained at 24°C. After obtaining three 24 h baseline recordings of S-W and T_{br} at 24°C, the mPOA neurons were destroyed by microinjection of NMDA (5 µg in 0.2 µl distilled water, neutralized with NaOH) injected following the De Groot (22) atlas at the coordinates for mPOA as A 7.8, H –1.5 and L 0.6 mm as reported earlier, also (3, 16, 18) was injected. NMDA was injected in the second site (opposite side), 30–35 mins after the administration on the first site. On the 28th day, after the lesion, S-W and T_{br} were recorded for 24 h at 24°C. The temperature of the environmental chamber was lowered to 18 ± 1°C, after finishing the 28th day recording, and the animals were maintained continuously in this T_a for four weeks. On the 1st, 7th, 14th, 21st, and 28th days, S-W and T_{br} were recorded for 24 h. At the end of the experiment, the animals were sacrificed under sodium pentobarbitone anaesthesia (50 mg/kg bw ip). The brain tissues were fixed with intra-cardiac perfusion of 10% formalin. The brains were removed and 10 ìm thick sections were processed for histological examination of the lesion site, with Cresyl violet stain (23). The neural damage due to NMDA injection was ascertained as described earlier (24). The results from five animals, in which the site of lesion was confirmed to be at the mPOA, are described here. In these rats three 24 h control recordings were taken before lesion and one after 28th days of the mPOA lesion, and five recordings on the 1st, 7th, 14th, 21st, and on 28th days when they were exposed to cold T_a. The Institutional Animal Ethics Committee approved all protocols.

The sleep records were divided into 30 s epochs and visually analysed on the computer. The wakefulness (W) was subdivided into active wakefulness (W1) and quiet wakefulness (W2). The sleep was

divided into slow wave sleep (SWS) and paradoxical sleep (PS). The SWS was subdivided into light slow wave sleep (S1) and deep slow wave sleep (S2). These S-W stages, total sleep time (TST), and the T_{br} that was noted at 30 s intervals, were quantified separately for 24 h and for light and dark periods. The durations and frequencies of different sleep episodes (S1, S2 and PS) were calculated. Friedman's non-parametric two-way analysis of variance test was used to find out the possible variation between the three control (pre-lesion) readings of S-W and T_{br}. The mean of the three pre-lesion readings were compared with the post-lesion data obtained on the 28th day using the same test. Post-Hoc test was used to find out the significance of each parameter.

The readings of S-W and T_{br} before cold exposure (the post-lesion data obtained on the 28th day) were compared with those obtained during the 1st, 7th, 14th, 21st and 28th days of cold exposure using Friedman's non-parametric two-way analysis of variance test and Post-Hoc test to find out the significantly changed, each, parameters. In addition, the data from this lesioned group were compared with the data of the non-lesioned group exposed to cold, which was reported earlier (4).

RESULTS

Effect of exposure of the mPOA lesioned rats to a mild cold T_a of 18°C on S-W and T_{br} are described here, along with the effect of the destruction of the mPOA neurons on these parameters. In addition, effects of exposure of these rats to 18°C were compared with the changes produced in the normal rats when they were exposed to the same mild cold T_a.

The three base line recordings of S-W and T_{br} of these five rats at 24°C, before the mPOA lesion were found to be comparable. W1 was the major component of W, whereas S1 formed the major component of the total sleep (Table I). The T_{br} was higher during the dark period than in the light period (Table II).

Histology

All the five rats included in this group had bilateral lesion in the mPOA. The destruction of the mPOA neurons produced by the injection of NMDA was restricted to the mPOA on both sides (Fig. 1). The bilateral destruction produced by the NMDA injection was in the mPOA, except for a very small mechanical destruction in the needle tracts.

Effect of lesion of the mPOA on S-W and T_{br}

All the components of sleep were decreased, and wakefulness increased during both light and dark periods when the recordings were taken after 28 days of the mPOA lesion (Table I). The episode durations of the different sleep stages i.e. S1, S2, and PS were decreased from the control level, whereas those of W1 and W2 were increased (Table III). The frequencies of the sleep episodes were also decreased, whereas those of the W1 and W2 were not significantly affected (Table III). The T_{br} was increased after the mPOA lesion during both the light and dark periods (Table II).

TABLE I: Different S-W stages (%) on different days during 24 h, light (14 h) and dark period (10 h) periods before lesion and in the mPOA lesioned rats before and after cold exposure (Mean±SD).

		W1	*W2*	*S1*	*S2*	*PS*
			Control			
	24 h	30.18±0.2	14.42±0.2	29.75±0.3	14.55±0.1	11.03±0.2
	Light, 14 h	29.71±0.4	14.11±0.4	30.45±0.3	14.82±0.1	11.17±0.3
	Dark, 10 h	30.83±0.3	14.86±0.63	28.76±0.9	14.17±0.2	10.82±0.2
			mPOA Lesioned			
	24 h	34.19±1**	17.00±0.9**	26.49±1.3**	12.80±0.5**	9.51±0.6**
	Light, 14 h	33.87±0.9**	16.92±0.82**	27.18±1.4**	13.10±0.5**	9.69±0.7**
	Dark, 10 h	34.65±1.4**	17.12±1.49**	25.53±1.1**	12.38±0.66**	9.27±0.6**
			Mild cold exposure (18°C)			
1st Day	24 h	39.44±3.0**≠	18.96±0.5**≠	22.13±0.9**≠	10.75±0.6**≠	7.58±0.4**≠
	Light, 14 h	39.00±3.2**≠	18.38±1.0*	22.77±0.7**≠	11.01±0.6**≠	7.86±0.5**≠
	Dark, 10 h	40.05±2.9**≠	19.77±1.1**≠	21.23±1.4**≠	10.38±0.8**≠	7.20±0.4**≠
7th Day	24 h	37.90±1.4**≠≠	16.99±0.9**	24.49±0.4**	11.76±0.7**	8.87±0.2**
	Light, 14 h	37.04±1.3**≠≠	16.60±1.0**	25.19±0.4**≠	12.04±0.8**	9.17±0.4**
	Dark, 10 h	39.10±2.0**≠	17.53±0.7**	23.52±0.7**≠	11.37±0.7**≠	8.45±0.1**≠
14th Day	24 h	35.78±1.0**	16.52±0.3**	25.65±0.9**	12.65±0.2**	9.40±0.3**
	Light, 14 h	35.27±1.0**	16.44±0.6**	26.29±0.7**	12.79±0.3**	9.71±0.1**
	Dark, 10 h	36.48±1.2**	16.63±0.3**	24.77±1.3**	12.47±0.3**	8.95±0.7**
21th Day	24 h	33.99±0.6**	16.15±0.3**	27.15±0.7**	12.95±0.3**	9.76±0.3**
	Light, 14 h	33.94±0.7**	15.83±0.4**	27.46±0.5**	13.13±0.4**	10.07±0.2**
	Dark, 10 h	34.05±0.6**	16.60±0.6**	26.72±1.02**	12.70±0.2**	9.32±0.4**
28th Day	24 h	33.94±0.8**	16.82±0.2**	27.06±0.3**	12.81±0.3**	9.36±0.3**
	Light, 14 h	33.62±0.7**	16.87±0.4**	28.04±0.4**	13.12±0.5**	9.49±0.4**
	Dark, 10 h	34.40±1.0**	16.75±0.2**	25.70±0.3**	12.38±0.2**	9.18±0.4**

**P≤0.01, ***P≤0.001, significantly different when compared with control; ≠P≤0.05, ≠≠P≤0.01, significantly different when compared with 28th Post-lesion day.

TABLE II: T_{br} during 24 h, light (14 h) and dark period (10 h) periods before lesion and in the mPOA lesioned rats before and after cold exposure (Mean ± SD).

	T_{br} (24 h)	*Light (14 h)*	*Dark (10 h)*
		Control	
	37.78±0.08	37.75±0.07	37.81±0.10
		mPOA Lesion	
	38.04±0.07**	38.03±0.08*	38.05±0.06**
		Mild cold exposure (18°C)	
1st Day	38.21±0.06**≠	38.20±0.04**≠	38.25±0.08**≠
7th Day	38.10±0.06**≠	38.09±0.07*≠	38.12±0.03**≠
14th Day	38.15±0.03**≠	38.13±0.07*≠	38.16±0.03**≠
21st Day	38.22±0.06**≠	38.20±0.04*≠	38.22±0.05**≠
28th Day	38.18±0.09**≠	38.15±0.07*≠	38.18±0.12**≠

**P≤0.01, ***P≤0.001, significantly different when compared with control; ≠P≤0.05, ≠≠P≤0.01, Significantly different when compared with 28th Post-lesion day.

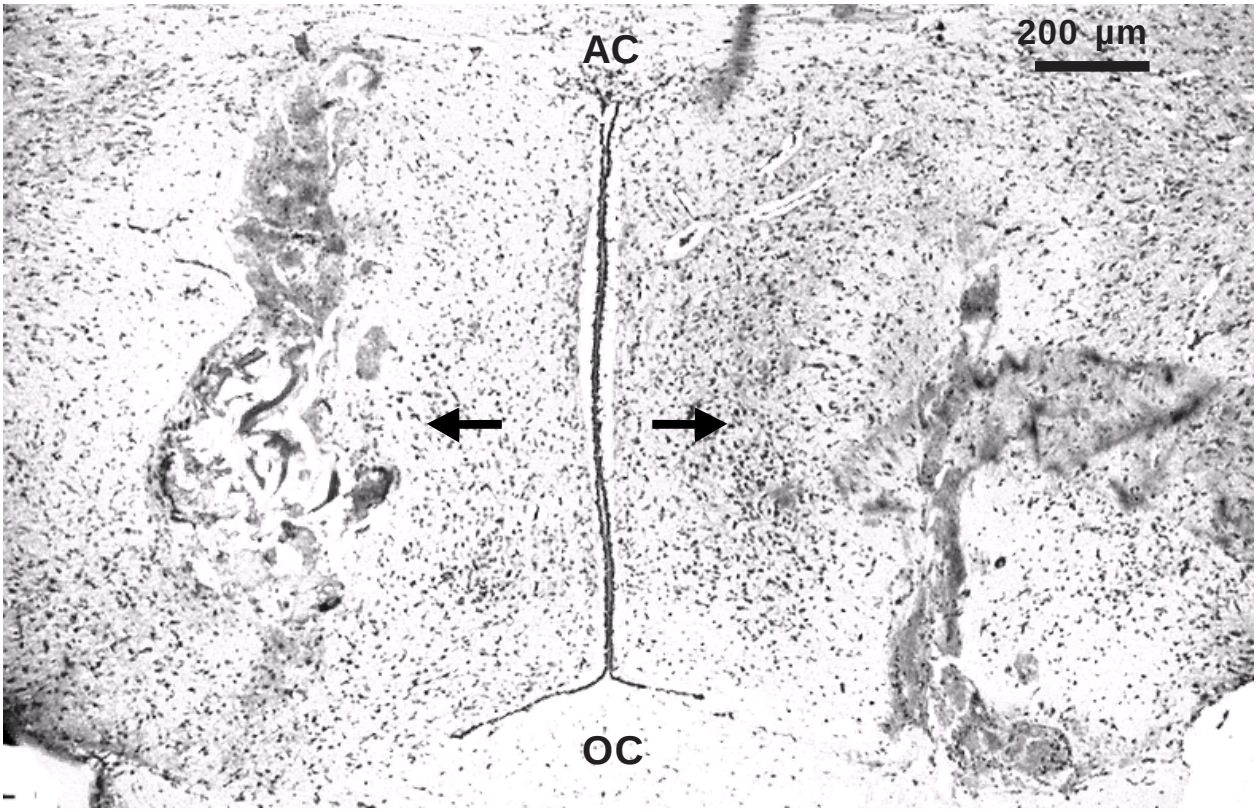

Fig. 1: Photomicrographs (Coronal section of 10 µm thick) of the brain, stained with Cresyl violet. The arrows point towards the area of neural damage in the mPOA due to the intracerebral injection of NMDA. AC–Anterior Commissure, OC–Optic Chiasma.

TABLE III: Episode duration (min) and frequency (per day) of different S-W stages in rats before and after the mPOA lesion.

	Episode Duration	
	Control	*28th Day Post Lesion*
W1	2.97±0.05	3.29±0.07^{++}
W2	2.05±0.01	2.53±0.04^{++}
S1	3.01±0.1	2.93±0.06^{-}
S2	2.13±0.09	1.98±0.07^{--}
PS	1.97±0.05	1.84±0.05^{--}
	Episode Frequency	
	Control	*28th Day Post Lesion*
W1	146.52±2.2	149.63±6.71
W2	101.43±5.72	96.97±6.43
S1	142.46±2.64	127.99±7.58^{-}
S2	103.03±1.6	91.57±4.06^{-}
PS	80.82±1.74	74.70±3.23^{-}

$^{++}$P≤0.01 significantly higher; $^{-}$P≤0.05 significantly lower; $^{-}$P≤0.01 significantly lower.

Effect of exposure of the mPOA lesioned rats to 18°C

When the mPOA lesioned rats were exposed to a mild cold T_a (18°C), on the 1st day, the TST and all the components of sleep were decreased and wakefulness increased from the pre-exposure levels (Table I, Fig. 2). The frequencies of S1, S2 and PS episodes were decreased, whereas the episode durations of these stages were increased (Fig. 3). The T_{br} was increased during light and dark periods on the 1st day of exposure to 18°C (Table II, Fig. 2).

The TST and the total quantity of different components of sleep, in the mPOA lesioned rats, came back to the pre-exposure

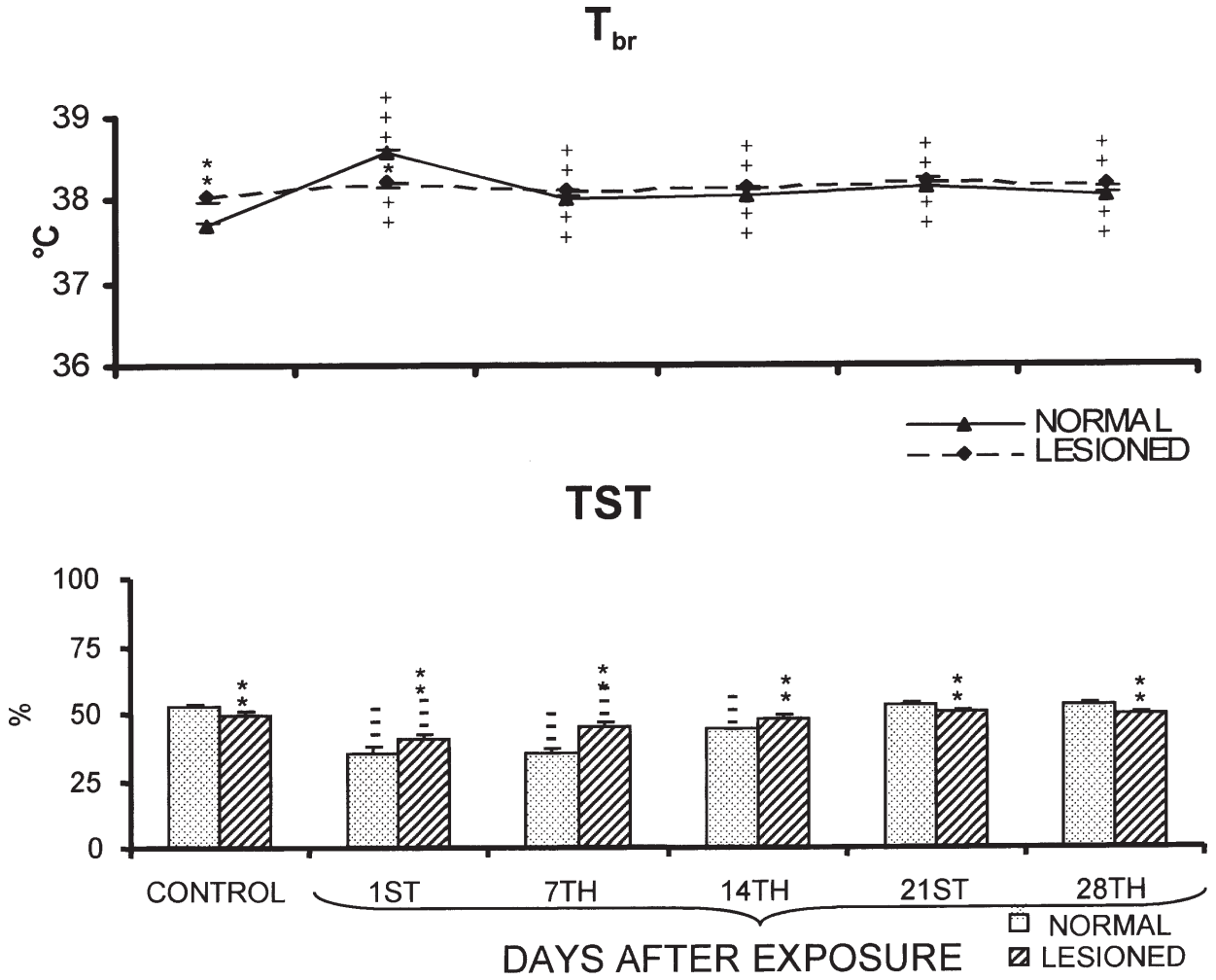

Fig. 2 : The line diagram in the upper panel shows the brain temperature (T_{br}) in °C on different days of cold (18 ± 1°C) exposure in normal and mPOA lesioned rats. The bar diagram in the lower panel shows the total sleep time (TST) in % during the same period. Previous work on normal rats (Ref. 4) is reproduced with permission. $^{++}P \leq 0.01$, $^{+++}P \leq 0.001$ significant increase from the control level. $^{---}P \leq 0.001$ significant decrease from the control level.

level by the 14th day of continued cold exposure (Table I, Fig. 2). On the other hand, the episode durations of the sleep stages were increased through out the period of cold exposure (Fig. 3). The increase in the episode durations was more pronounced during the later days of exposure. An increase in the episode durations was responsible for the recovery of the total sleep by 14th day. However, the frequency of the sleep episodes remained low, throughout the period of cold exposure. The increase in T_{br} persisted throughout the cold exposure in the mPOA lesioned rats. This increase in the T_{br} was seen both during light and dark periods.

Comparison of the changes in S-W and T_{br} between normal (non-lesioned) and the mPOA lesioned group of animals after exposure to cold

Though the TST of the lesioned rats was decreased on the 1st and 7th days of cold

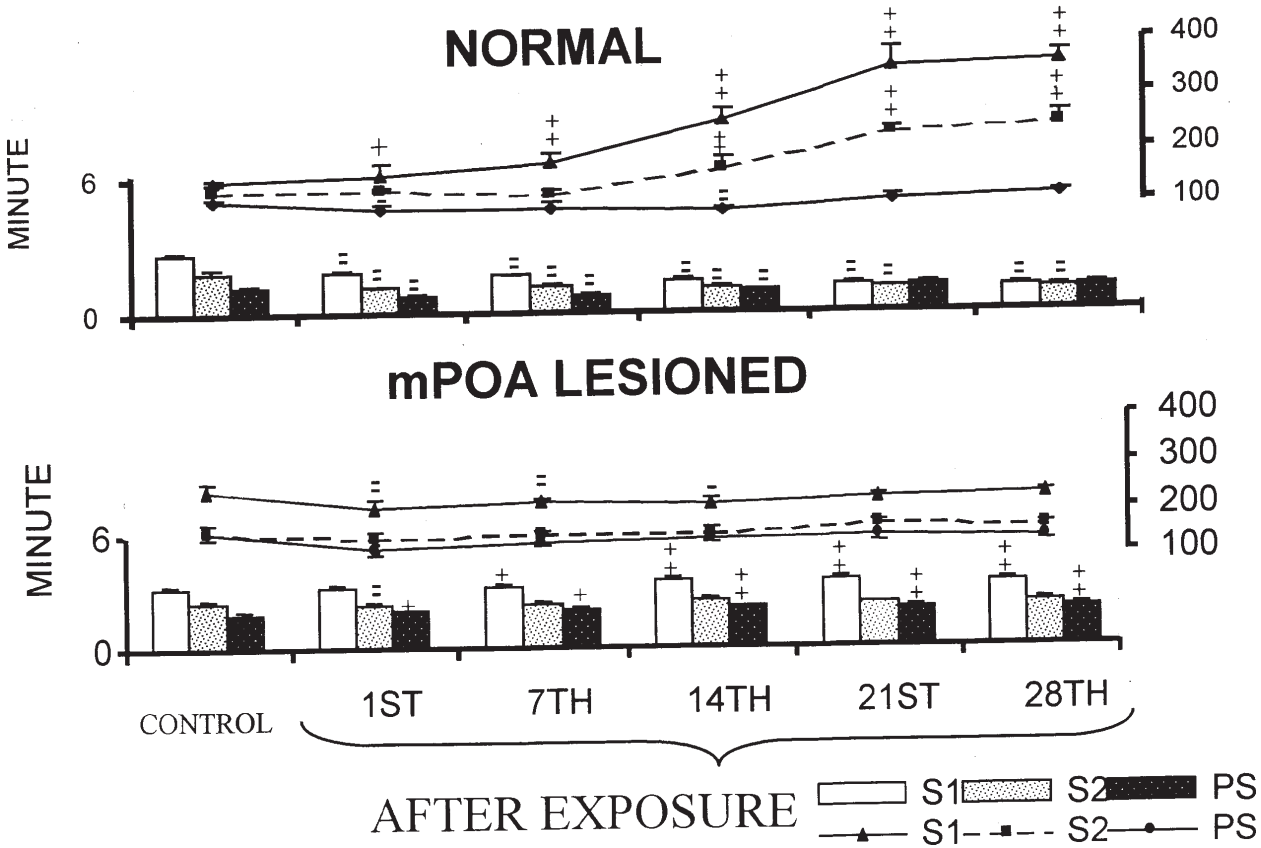

Fig. 3 : The upper panel shows the distribution of frequencies and durations of episodes of S1, S2, and PS before exposure (control), and during 1st, 7th, 14th, 21st and 28th days of cold exposure (18 ± 1°C). The lower panel shows the distribution of frequencies and episode durations before and after exposure to cold (18 ± 1°C) in mPOA lesioned rats. The frequencies are shown in lines and the episode durations in bars. In the lower panel, in the secondary Y-axis the maximum value (200) is different from that of the upper panel (400).
$^{+}P \leq 0.05$, $^{++}P \leq 0.01$ significant increase from the control level.
$^{-}P \leq 0.05$, $^{--}P \leq 0.001$ significant decrease from the control level.
*P≤0.05, significantly different from the corresponding day's value of normal group.

exposure, this reduction was much less than in the normal rats (Fig. 2). The frequencies of S1, S2 and PS episodes were not only lowered, but also remained lower than that of the non-lesioned group. On the other hand, increase in frequencies of sleep episodes was responsible for sleep recovery in the non-lesioned rats.

The base line T_{br} of the lesioned group was found to be significantly higher than that of the non-lesioned group (Fig. 2). Though the T_{br} was further increased on the 1st day of exposure to cold, the T_{br} levels attained in the lesioned animals were found to be significantly less than the value obtained in the non-lesioned group. But, during chronic exposure there was no significant difference between the lesioned and non-lesioned groups.

DISCUSSION

The effects of exposure to cold T_a on S-W and T_{br} were studied on the rats after 28 days of the mPOA lesion. Though the

decrease in sleep and the increase in T_{br}, during the initial days of cold exposure, were similar to that observed in normal rats, the magnitude of changes were much less in the lesioned rats (4). Increase in sleep episode durations was responsible for the recovery of sleep in the lesioned rats, but an increase in the frequency of sleep episodes was responsible for sleep restoration in the normal rats.

The values of various components of S-W and T_{br} before the mPOA lesion, when the rats were maintained at 24°C are comparable to the earlier reports (4, 16, 25–27). Acute cold exposure produced decrease in sleep in the mPOA lesioned rats (3). During continued exposure, sleep was restored to the pre-exposure level in the normal rats (4). This phenomenon was also observed in the mPOA lesioned rats. Therefore, the rats with damage to the mPOA neurons do have the neural mechanism, which is capable of increasing their quantum of sleep that was reduced by cold exposure. It also shows that the lower level of sleep, attained after the mPOA lesion is the new homeostatic level, which is defended whenever it is decreased from this equilibrium.

Though the T_{br} was higher in the mPOA lesioned rats, it was further increased during chronic cold exposure. At the same time, it was also seen that the magnitude of rise in T_{br} especially during the initial days of cold exposure was lower in the mPOA lesioned rats. So, the mPOA lesion did affect the magnitude of responses of T_{br}, though it did not abolish the responses completely. On the basis of this observation, it can be concluded that the mPOA plays a role in the cold induced changes in T_{br}. It was earlier suggested that the increase in T_{br} might be contributing towards the restoration of sleep during chronic cold exposure (4). It has been demonstrated that even a mild warming of the thermosensitive areas of the basal forebrain produces increase in sleep (5, 6). So, the resetting of thermostat at a higher level by the physiological adaptive mechanism may be also aimed at restoring sleep during cold exposure.

It cannot be ruled out that a decrease in the cold induced acute changes in S-W in the mPOA lesioned rats could also be due to a decrease in the stress response of these animals. Though 18°C is not much of a stress for rats, the lesioned rats were probably less stressed by the cold than the normal animals. So, the increase in wakefulness was less in the lesioned rats during the initial days of cold exposure. As wakefulness is associated with an increase in T_{br} (6,18, 25, 29–31), the reduced magnitude of increase in wakefulness may be responsible for the reduced rise in T_{br}.

The decreases in sleep and increase in T_b/T_{br} after the mPOA neuronal destruction was similar to the earlier reports (16, 18, 21, 25, 26, 31, 32). It was suggested that the thermostat of the thermoregulatory mechanism was reset at a higher level in these lesioned rats (16, 18, 21). Further support for this hypothesis came from the earlier observation that these rats voluntarily selected a warmer environment after the mPOA lesion (21). Hyperthermia was not due to a failure of heat dissipating mechanism as the mPOA lesioned rats were shown to be capable of defending their body temperature when they were exposed to a very hot environment (17). So, it is reasonable to conclude that the elevation in T_{br} was due to resetting of thermostat at a higher level, rather than to a failure of the

heat dissipating mechanism, as was suggested earlier (14).

The lesion of the mPOA resulted in a decrease in the duration of sleep episodes. On the basis of this observation, it was earlier concluded that the primary role of the mPOA is sleep maintenance (16, 18). As the durations of sleep episodes were increased during cold acclimatization in the mPOA lesioned rats, this assumption has to be modified or clarified. It should therefore be assumed that the sleep requirement had come down in the mPOA lesioned rats. The reduction in sleep requirement would have had its effect on the frequency and duration of sleep episodes. But, the deficiency on sleep initiation (frequency) was not visible in rats maintained in a normal (or warm) environment. The increase in sleep pressure, caused by the cold induced sleep loss, resulted in a rebound increase in the duration of sleep episodes along with a decrease in the frequency of sleep episodes. This showed that the sleep pressure could cause an increase in the duration of sleep episodes. Decrease in the frequency of sleep episodes brought out clearly the deficiency in sleep initiation in the mPOA lesioned rats.

It was stated earlier that the primary role of the mPOA is to interlink various physiological regulations (16). After the mPOA lesion, the rats were unable to interlink energy homeostasis with sleep homeostasis. It was also shown earlier that the rats with the mPOA lesion showed a reduction in body weight, as they were not increasing their energy intake even when the energy expenditure had increased. The present findings, in fact, further support this hypothesis. After the mPOA lesion, the rats are less capable of increasing their T_{br}. Adjustment of body temperature or T_{br} could be considered as a part of energy homeostasis. The ability of the rats to increase their T_{br} was affected after the mPOA lesion. Increasing the T_{br} would have helped in dealing with the disturbed in sleep homeostasis.

Even though the homeostatic mechanism is affected in the mPOA lesioned rats, it is not completely abolished. The rest of the brain including the left over mPOA had the ability to increase the quantity of sleep. The method employed by the mPOA lesioned and normal rats were different. The normal rats increased their sleep episodes to recover from the induced reduction in sleep (4). But, the mPOA lesioned rats recovered it by increasing the durations of sleep episodes.

The T_{br} was increased not only during chronic cold exposure, but also after the mPOA lesion. So, it could be suggested that the resetting of T_{br} is a physiological readjustment aimed at restoration of sleep. The effects of cold T_a was studied after four weeks of the mPOA lesion, as earlier studies have shown that the S-W and T_{br} were stabilised at the new level by this time (16).

REFERENCES

1. Schmidek WR, Hoshino K, Schmidek M. Timo-Iaria C. Influence of environmental temperature on the sleep-wakefulness cycle in the rat. *Physiol Behav* 1972; 8:363–371.

2. Parmeggiani PL, Agnati LF, Zamboni G and Cianci T. Hypothalamic temperature during the sleep cycle at different ambient temperatures. *Electroencephalogr Clin Neurophysiol* 1975; 38: 589–596.

3. Thomas TC and Kumar VM. Role of medial preoptic neurons in the regulation of sleep and brain temperature under different ambient temperatures. *Sleep Res Online* 2000; 3: 141–145.

4. Mahapatra APK, Mallick HN and Kumar VM. Changes in sleep on chronic exposure to warm and cold ambient temperatures. *Physiol Behav* 2005; 84: 284–291.

5. Roberts WW and Robinson TCL. Relaxation and sleep induced by warming of the preoptic area and anterior hypothalamus in cats. *Exp Neurol* 1969; 25: 282–294.

6. Roberts WW, Bergquist EH, and Robinson TC. Thermoregulatory grooming and sleep-like relaxation induced by local warming of preoptic area and anterior hypothalamus in opossum. *J Comp Physiol Psychol* 1969, 67: 182–188.

7. McGinty D, Szymusiak R, Thomson D. Preoptic/anterior hypothalamic warming increases EEG delta frequency activity within non-rapid eye movement sleep. *Brain Res* 1994, 26; 667: 273–277.

8. Glotzbach SF and Heller HC. Central nervous regulation of body temperature during sleep. *Science* 1976; 194: 537–539.

9. Parmeggiani PL, Zamboni G, Cianci T, Agnati LF and Ricci C. Influence of anterior hypothalamic heating on the duration of fast wave sleep episodes. *Electroencephalogr Clin Neurophysiol* 1974; 36: 465–470.

10. Parmeggiani PL and Rabini C. Sleep and environmental temperature. *Arch Ital Biol* 1970; 108: 369–387.

11. Heller HC and Glotzbach SF. Thermoregulation during sleep and hibernation. *Int Rev Physiol* 1977; 15: 147–188.

12. Haskell EH, Palca JW, Walker JM, Berger RJ and Heller HC. The effects of high and low ambient temperatures on human sleep stages. *Electroencephalogr Clin Neurophysiol* 1981; 51: 494–501.

13. Rosenthal MS and Vogel GW. The effect of a 3-day increase of ambient temperature toward the thermoneutral zone on rapid eye movement sleep in the rat. *Sleep* 1993; 16: 702–705.

14. Szymusiak R and Satinoff E. Ambient temperature dependence of sleep disturbances produced by basal forebrain damage in rats. *Brain Res Bull* 1984; 12: 295–305.

15. John J and Kumar VM, Gopinath G, Ramesh V and Mallick HN. Changes in sleep–wakefulness after kainic acid lesion of POA in rats. *Jpn J Physiol* 1994; 44: 231–242.

16. John J and Kumar VM. Effect of NMDA lesion of medial preoptic neurons on sleep and other functions. *Sleep* 1998; 21: 585–597.

17. Kumar VM and Khan NA. Role of the medial preoptic neurons in thermoregulation in rats. *Arch Clin Exp Med* 1998; 7:24–27.

18. Thomas TC and Kumar VM. Effect of ambient temperature on brain temperature and sleep-wakefulness in medial preoptic area lesioned rats. *Indian J Physiol Pharmacol* 2002; 46: 287–297.

19. Lipton JM, Dwyer PE and Fossler DE. Effects of brainstem lesion on temperature regulation in hot and cold environments. *Am J Physiol* 1974; 226: 1356–1365.

20. Satinoff E, Liran J and Clapman R. Aberrations of circadian body temperature rhythms in rats with medial preoptic lesions *Am J Physiol* 1982; 242: R352–R357.

21. Ray B, Mallick HN and Kumar VM. Changes in sleep-wakefulness in the medial preoptic area lesioned rats: Role of thermal preference. *Behav Brain Res* 2004.

22. DeGroot J. The rat forebrain in stereotaxic coordinates. Verh Konink Nederland Akad Wetenschap Natuurkunde 1959; 52: 1–40.

23. Drury RAB and Willington EA. The nervous system. In: Carleton's histological techniques. Oxford Univ Press 1980; 354–390.

24. Kohler C and Schwarcz R. Comparison of ibotenate and kainate toxicity in the rat brain: a histological study. *Neuroscience* 1983; 8: 819–835.

25. Alfoldi P, Rubicsek G, Cserni G and Obal Jr F. Brain and core temperature and peripheral vasomotion during sleep and wakefulness at various ambient temperatures in the rat. *Pflugers Arch* 1990; 417: 336–341.

26. Szymusiak R and McGinty D. Sleep related neuronal discharge in the basal forebrain of cats. *Brain Res* 1986; 370: 82–92.

27. Ramesh V and Kumar VM. The role of alpha-2 receptors in the medial preoptic area in the regulation of sleep-wakefulness and body temperature. *Neuroscience* 1998; 85: 807–817.

28. Sterman MB and Clemente CD. Forebrain inhibitory mechanism: sleep patterns induced by basal forebrain stimulation in the behaving cat. *Exp Neurol* 1962; 6: 103–117.

29. Obal, F Jr, Tobbler I. and Borbely A A., Effect of ambient temperature on 24h sleep-wake cycle in normal and capsaicin treated rats. *Physiol Behav* 1983; 30: 425–430.

30. Franken P,Tobler I and Borbely AA. Cortical temperature and EEG slow wave activity in the rat: analysis of vigilance state related changes. *Pflugers Arch* 1992; 420: 500–507.

31. Gao B, Franken P, Tobler I and Borbely AA. Effect of elevated ambient temperature on sleep, EEG spectra, and brain temperature in the rat. *Am J Physiol* 1995; 268: R1365–R1373.

32. Palaca JW, Walker JM and Berger RJ. Thermoregulation, metabolism and stages of sleep in cold-exposed men. *J Appl Physiol* 1986; 61: 940–947.

33. Szymusiak R, Danowski J and McGinty D. Exposure to heat restore the sleep in cats with preoptic/anterior hypothalamic cell loss. *Brain Res* 1991; 541: 134–138.