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WARM SENSITIVE NEURONS OF THE PREOPTIC AREA REGULATE AMBIENT TEMPERATURE RELATED CHANGES IN SLEEP IN THE RAT

DEEPENDRA KUMAR[a,b], VELAYUDHAN MOHAN KUMAR[a,b] AND HRUDA NANDA MALLICK[a]*

[a]*Department of Physiology, All India Institute of Medical Sciences, New Delhi – 110 029, India*

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Abstract : Warm sensitive neurons (WSN) play a major role not only in body temperature regulation, but also in sleep regulation. The present study was undertaken to investigate the role of WSN of the preoptic area (POA) in mediating the ambient temperature (T_{amb}) related changes in sleep. The effect of T_{amb} changes on sleep and body temperature was studied in rats before and after destruction of WSN of the POA by local intracerebral injection of capsaicin. Though the rats preferred 27°C T_{amb}, they slept maximum at 30°C. After destruction of WSN of the POA, slow wave sleep (SWS) peak was brought down to 27°C, which was the preferred T_{amb} of the rats. This indicates that WSN of the POA mediate the increase in SWS, at temperatures higher than preferred T_{amb}. On the other hand, in WSN destroyed rats, rapid eye movement (REM) sleep was maximum at 33°C. It suggests that the REM sleep generation is under inhibitory control of the WSN of the POA. The study supports several earlier reports that the neurons of the POA play a key role in coordinating sleep and body temperature regulation.

Key words : capsaicin, ambient temperature, preoptic area, sleep, warm sensitive neurons, body temperature, thermal preference

INTRODUCTION

The preoptic area (POA) of the basal forebrain is the most important neural site for thermoregulation (1-11). The POA is also an important brain area for sleep regulation, and several reports suggest that the interlink between sleep and thermoregulation is mediated through this brain area (6, 7, 12–22). One important observation in support of this interlink was the increase in slow wave sleep (SWS) and rapid eye movement

*Corresponding Author : Dr. Hruda Nanda Mallick, Professor, Department of Physiology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi – 110 029, India; Tel.: + 91-11-26594881; Fax: +91-11-26588641, +91-11-26588663; E-mail: drhmallick@yahoo.com

[b]Present address : Comprehensive Centre for Sleep Disorders, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram – 695 012, India; Tel.: +91-471-2328004; Fax : +91-471-2341814.

(REM) sleep on exposure to moderately high ambient temperature (T_{amb}) (23–28). It is interesting to note that the T_{amb}, which produce maximum sleep, is actually higher than the temperature that the rats prefer (27, 28). In fact, the increased sleep persisted, even when the rats were kept continuously in that high T_{amb} for four weeks (29). The T_{amb} which produce an increase in sleep in rats, does also produce a slight increase in the body temperature (T_b) (30). Increased T_b would stimulate thermoreceptors throughout the body (31–34). The influence of T_{amb} on sleep was suggested to be mediated by peripheral and central warm receptors, as this response was abolished when all the warm receptors and central warm sensitive neurons (WSN) were destroyed by systemic administration of capsaicin (35). But it was later shown that the increase in total sleep time (TST), with increasing T_{amb}, occurred even after selective destruction of the peripheral warm receptors (27). Although these studies indicate the relative importance of central thermoreceptors in sleep regulation, they failed to pinpoint the location of the central receptors that were primarily responsible for this response. The POA, which has a large number of WSN, is likely to play an important role not only in thermoregulation but also in sleep regulation (36–39). As the TST could also be increased by warming the POA (40), the increase in sleep with changing T_{amb} is probably mediated by the WSN of this brain area. In order to prove this hypothesis, there is a need to study the influence of T_{amb} on sleep in animals in which WSN of the POA are destroyed. Capsaicin, an active substance of hot peppers, can selectively destroy the WSN without any apparent effect on cold receptors. In the present study, the effect of wide-ranging T_{amb} on sleep in rats was investigated before and after destruction of WSN of the POA, by local intracerebral injection of capsaicin. Changes in T_b were also monitored along with sleep. The T_{amb} preferred by the rats was also assessed before the animals were selected for the study.

MATERIAL AND METHODS

Sleep-Wakefulness (S-W) and T_b were recorded on rats with chronically implanted electrodes and telemetric transmitter (19, 28). Their T_{amb} preference was assessed in an environmental chamber, specially designed for this purpose (28, 41, 42). The S-W stages and T_b of these rats were studied when they were exposed to different T_{amb}, ranging between 18° and 36°C T_{amb}, before destruction of WSN in the POA. Functional integrity of warm thermoreceptors and WSN in these rats were assessed by using the capsaicin sensitivity test (43–45). After control recordings, high dose of capsaicin was injected intracerebrally to destroy the WSN of the POA (43, 44, 46–49). Capsaicin sensitivity test and thermal preference assessment were again done after destruction of the WSN. Comparing the changes in S-W and T_b at various T_{amb}, before and after intracerebral injection of capsaicin, provided information about the role of WSN in the POA in sleep regulation.

Six adult male Wistar rats, weighing between 250–275 g each, were used for this study. They were housed individually in transparent polyethersulphone cage (floor, 445 × 295 × 185 mm and cage cover set, 450 × 295 × 140 mm) with controlled temperature and *ad libitum* access to food and water (Millennium SPF Rack System R-

series, Orient Co.LTD, South Korea). The rats were maintained at 14 h light (illumination above 200 lux) and 10 h dark (illumination below 5 lux) conditions with light on from 06:00 h. Implantation of electrodes was conducted under thiopentone sodium, THIOSOL anesthesia (40 mg/kg body weight, I.P.). Electrodes for electroencephalogram (EEG), electromyogram (EMG) and electrooculogram (EOG) were chronically implanted and connected to a plug that was fixed to the skull, as described elsewhere (50). For the intracerebral injection of capsaicin at the POA (after the control recordings) two tiny holes were drilled on the skull, 0.6 mm lateral to the mid sagittal suture and 2 mm anterior to the bregma (51).

Radio-transmitters, TA10TA-F40 (Data Science International, USA), were implanted in the abdomen of the animals (19). All procedures were conducted in accordance with the rules of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and were approved by the Institutional Animal Ethics Committee, AIIMS, New Delhi, India. After a ten-day recovery period, the rats were trained for two days to move freely in the recording cage with the attached cable. Flexible cables with connectors were plugged to the rats' heads. Output from the plug was connected to BIOPAC system Inc (BSL PRO 36, USA) for digital recordings of EOG, EEG, and EMG, along with telemetric recording of T_b (DATA QUEST 1.1, Data Science International, and USA) on seven alternate days.

The thermal preference of the rats was studied in an environmental chamber with three compartments, fitted with activity monitoring systems (Habitest response sensing module, model E45-04, Coulbourn Instrument, USA), as described earlier (41). Vibration-sensing platforms were kept below the floors of all the compartments of the environmental chamber. The system measured ergometric activity by converting the animals' displacement of mass into pulses representing units of time-integrated load displacement. The signals were sent through Habitest Linc interface (l91-04HS) to a computer, where the software analyzed the signals in terms of beginning of mass displacement of one compartment, and end of displacement in the other. Thus the software assessed the time spent by the animals in each of the compartments. The three compartments of the chamber were maintained at 27°, 30°, and 33°C respectively. These three T_{amb} were selected on the basis of existing literature; 27°C being the T_{amb} preferred by the rats as per earlier report (28,41-42) and 30°, and 33°C being the temperatures shown to induce maximum sleep (26-28).

After assessment of thermal preference, S-W and T_b were recorded for 2 h (9:00-11:00 h) at 27°C, and for subsequent 6 h (11:00-17:00 h) with T_{amb} maintained at 18°, 21°, 24°, 27°, 30°, 33° and 36°C. Gap of a day was given between the recordings. The temperature regulating system of the recording cage was capable of obtaining the altered T_{amb} in the recording box within five minutes. The conditions of the recording cage were kept identical to that of the thermal preference recording chamber, except for T_{amb}, which varied on different days of exposure. The relative humidity varied from 70 and 80% between the lowest and highest temperatures

respectively inside the cage.

After control recordings of thermal preference, and S-W and T_b at various T_{amb}, capsaicin sensitivity test was done. This was done by injecting capsaicin (8-methyl-N-vanillyl-6-nonenamide, Sigma, USA) subcutaneously at a dose of 2 mg/kg body weight and measuring the fall in T_b. This capsaicin induced change in T_b was again recorded after two weeks of destruction of WSN of the POA.

The head of the rat was fixed in a stereotaxic apparatus, under pentobarbitone sodium anaesthesia (30 mg/kg), for intrapreoptic injection of capsaicin to destroy the WSN of this area. 1% stock solution of capsaicin in a solvent containing 10% ethanol, 10% Tween 80 in 80% isotonic saline (52) was diluted before injection with sterile pyrogen-free saline to prepare a solution containing 5 µg/µl capsaicin. A 26-gauge sterile injector cannula was lowered into the POA (coordinates A 7.8, H 0.5 and L 0.6 mm as per De Groot atlas) through the pre-drilled holes on the skull, and capsaicin (25 µg in 5 µl) was slowly and repeatedly injected bilaterally, five times, at intervals of 30 to 45 minutes.

The T_b values during capsaicin sensitivity test after destruction of WSN of the POA were compared with data obtained before lesion, using Wilcoxon Signed Rank Test.

The S-W records were split into 15 s epochs and visually classified on the basis of EEG, EMG and EOG, as described earlier (53, 54). The data of all the parameters obtained from the six rats during the 2 h recordings during 9:00-11:00 h at 27°C on seven days were compared, by using Kruskal-Wallis H-test, to find out their variability. As there was no significant variation between the data obtained on the different days, it was assumed that the exposure to different temperatures, and the order of exposure on alternate days, did not have any spill over effect on pre-exposure data recorded for 2 h.

The data obtained for 6 h at 11:00-17:00 h at 27°C were taken as the control reading for comparison (using Wilcoxon Signed Rank Test) with the data obtained at corresponding timings at 18°, 21°, 24°, 30°, 33° and 36°C. The 6 h data, from two consecutive T_{amb} were also compared using the same test.

The 6 h data of S-W stages and T_b at different T_{amb} after the destruction of WSN of the POA were compared with data obtained before destruction, using Wilcoxon Signed Rank Test.

RESULTS

The subcutaneous injection of 2 mg/kg of capsaicin in normal rats produced a fall in T_b. A fall of about 3°C was observed after an hour of injection, and it persisted for about an hour. The same dose of capsaicin produced a lower fall in T_b after destruction of WSN, though the trend of change remained almost similar (Fig. 1).

The normal rats preferred to stay at 27°C, during 9:00-17:00 h, when they were provided a choice of three different temperatures of 27°, 30°, and 33°C (Fig. 2). The thermal preference of these rats did not change after destruction of WSN of the POA.

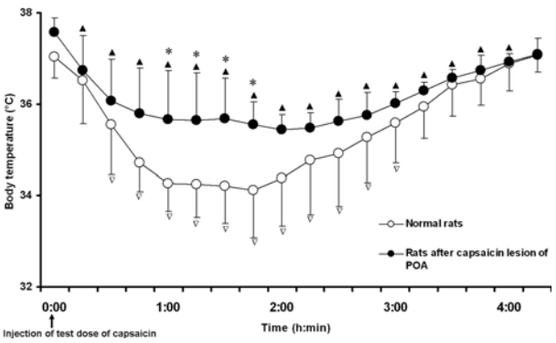

Fig. 1: The figure shows body temperature (mean±SD) changes in rats (n=6) to subcutaneous injection of capsaicin (2 mg/kg body weight), before and after destruction of preoptic warm sensitive neurons. Y-axis – body temperature in degree Celsius. X-axis- recording time. The arrow indicate the time point (0:00) of injection.

*P<0.05, comparison of T_b value after lesion of POA with its value recorded before lesion.

∇P<0.05, comparison of T_b values after test dose injection with its normal (preinjection) T_b value in normal rats.

▲P<0.05, comparison of T_b values after test dose injection with its normal (preinjection) T_b value in lesioned rats.

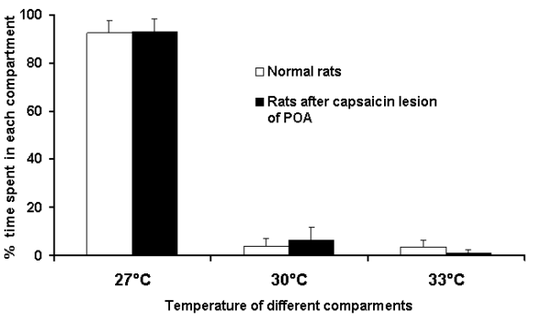

Fig. 2: The figure shows thermal preference of rats. Y-axis shows the time of stay (mean±S.D.) in each compartment, before and after destruction of preoptic warm sensitive neurons. X-axis – temperatures in the three compartments.

Though the normal rats preferred to stay at 27°C, the maximum sleep was recorded when they were kept at 30°C (Fig. 3). There was a decrease in TST above and below this

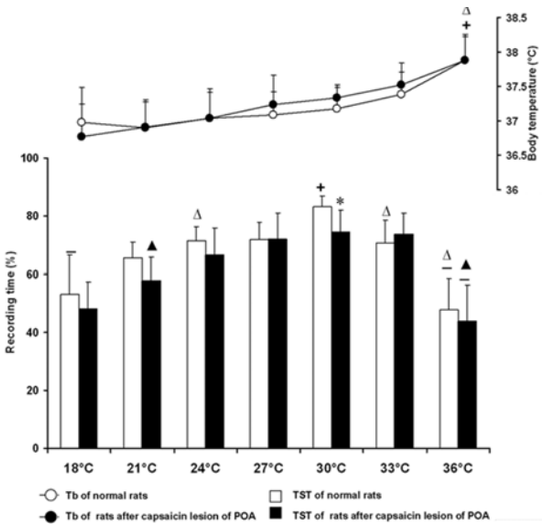

Fig. 3: The figure shows the mean±S.D. of total sleep time (bar diagram) and body temperature (line diagram) of rats (n=6) before and after destruction of preoptic warm sensitive neurons when they are exposed to various ambient temperatures. X-axis shows the ambient temperatures. Y-axis shows percentage recording time (bar diagram) and body temperature (line diagram).

–P<0.05, significant decrease compared to 27°C.

+P<0.05, significant increase compared to 27°C.

*P<0.05, significance of change in POA lesioned rats compared to normal at the same temperature.

ΔP<0.05, significant change in 24°C compared to 21°C, in 33°C compared to 30°C, and in 36°C compared to 33°C in total sleep time, and 36°C compared to 33°C in body temperature.

▲P<0.05, significant change in 21°C compared to 18°C, and in 36°C compared to 33°C.

temperature, with a steep decrease in sleep at higher T_{amb}. Though SWS and REM sleep followed the same trend as the TST, changes in REM sleep were more marked and significant (Fig. 4). Destruction of WSN of the POA did alter these trends of changes. The decrease in TST at 33°C, observed in normal rats, was not seen after WSN destruction. In fact, REM sleep was maximum at 33°C in the rats with destroyed

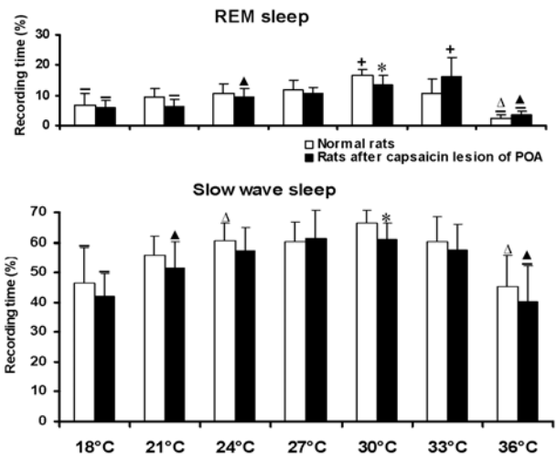

Fig. 4: The figure shows REM sleep and slow wave sleep at various ambient temperatures in rats (n=6) before and after destruction of preoptic warm sensitive neurons. X-axis shows the ambient temperatures. Y-axis shows percentage of REM sleep and slow wave sleep.

–P<0.05, significant decrease compared to 27°C

+P<0.05, significant increase compared to 27°C.

*P<0.05, significance of change in POA lesioned rats compared to normal at the same temperature.

ΔP<0.05, significant change in REM sleep in 36°C compared to 33°C, and in slow wave sleep in 24°C compared to 21°C, and in 36°C compared to 33°C.

▲P<0.05, significant change in REM sleep in 24°C compared to 21°C and in 36°C compared to 33°C, and in slow wave sleep in 21°C compared to 18°C, and in 36°C compared to 33°C.

WSN. Increase in the duration of REM episodes was responsible for this increase in REM sleep (Tables I and II).

The SWS peak at 30°C was not observed after the destruction of WSN of the POA. Not only was the SWS generally low after the destruction of WSN, even its peak had apparently shifted to 27°C, which was the preferred temperature of the rats.

TABLE I: Durations (min/h) of S-W episodes (mean±S.D) at various ambient temperature before (control) and after destruction of preoptic warm sensitive neurons (capsaicin) of rats.

Ta		*Wake*	*Slow wave sleep*	*REM sleep*
18°C	Control	2.35±0.7	1.18±0.2*	0.96±0.3*
	Capsaicin	3.49±0.4*▲	1.37±0.2	1.12±0.4
21°C	Control	4.72±0.3*	1.39±0.2	1.38±0.5
	Capsaicin	5.30±0.6*▲	1.20±0.3	1.05±0.3
24°C	Control	4.37±0.7*	1.51±0.3	1.34±0.3
	Capsaicin	4.15±0.7*	1.11±0.2	1.55±0.4
27°C	Control	2.52±0.3	1.55±0.3	1.63±0.3
	Capsaicin	2.13±0.3▲	1.39±0.3	1.13±0.3▲
30°C	Control	0.97±0.3*	1.35±0.1	1.51±0.1
	Capsaicin	2.04±0.5▲	1.14±0.1▲	1.28±0.3
33°C	Control	1.98±0.4*	1.51±0.3	0.85±0.3*
	Capsaicin	2.42±0.4▲	1.26±0.2	1.78±0.4*▲
36°C	Control	2.61±0.4	1.15±0.2*	0.54±0.2*
	Capsaicin	4.77±1.2*▲	1.00±0.2*	0.51±0.2*

*P<0.05, significance of change at different temperature compared to 27°C. ▲P<0.05, significance of change in rats after destructions of preoptic warm sensitive neurons compared to before (normal).

TABLE II: Frequency (h^{-1}) of S-W episodes (mean±S.D.) at various ambient temperature before (control) and after destruction of preoptic warm sensitive neurons (capsaicin) of rats.

Ta		*Wake*	*Slow wave sleep*	*REM sleep*
18°C	Control	70.0±9.0*	145.2±19.3	24.3±8.2
	Capsaicin	53.3±13.8	110.3±7.4*▲	23.2±6.2
21°C	Control	27.8±3.2*	144.2±16.2	22.3±7.1
	Capsaicin	27.7±9.5*	165.5±21.6	20.7±4.4*
24°C	Control	24.3±4.0*	143.2±12.2	29.3±7.4
	Capsaicin	29.0±6.5*	187.5±30.7▲	22.8±7.1*
27°C	Control	39.2±5.8	137.5±13.4	30.3±3.8
	Capsaicin	47.0±12.4	162.8±26.4▲	34.0±6.5
30°C	Control	65.7±13.6*	177.8±17.1*	39.7±3.1*
	Capsaicin	45.2±5.8▲	191.8±10.6*	39.2±10.2
33°C	Control	54.2±11.4*	152.5±22.7	33.0±5.4
	Capsaicin	40.2±12.4	166.0±23.9	33.3±12.7
36°C	Control	71.5±7.8*	142.7±23.1	16.0±5.5*
	Capsaicin	42.3±11.5▲	145.5±33.7	26.2±11.6

*P<0.05, significance of change at different temperature compared to 27°C. ▲P<0.05, significance of change in rats after destructions of preoptic warm sensitive neurons compared to before (normal).

The T_b of rats showed increasing and decreasing trend at T_{amb} above and below 27°C, both in normal and in WSN-lesioned rats (Fig. 3).

DISCUSSION

The preferred T_{amb} (i.e. 27°C) of the rats was the same before and after destruction of the WSN of the POA. Normal rats showed increase in SWS, at higher than preferred T_{amb}. This response was not observed after destruction of WSN of the POA. On the other hand, REM sleep showed an increase at higher T_{amb}, and it was maximum at 33°C, in the WSN destroyed rats.

Increases in TST, SWS and REM sleep, observed in this study in normal rats, when they were exposed to mild warm T_{amb}, are similar to the earlier reports (23-28). Sleep was highest at 30°C and it decreased above and below this T_{amb}. These increases in sleep parameters, at higher than preferred T_{amb}, can be assumed to be produced by stimulation of peripheral warm receptors and central WSN. When both the peripheral warm receptors and the central WSN were destroyed, increase in sleep with increased T_{amb} does not take place (35). On the other hand, sleep was increased at high T_{amb}, even after the peripheral warm receptors were selectively destroyed (27). This shows that the central WSN mediate the warm T_{amb} related increase in SWS and REM sleep.

Approximately one third of the POA neurons are warm sensitive (55). Though WSN are present in large number at the POA, they are not restricted to the POA (55). The present study was undertaken to investigate the role of WSN of the POA in mediating the warm T_{amb} related increase in SWS and REM sleep. Results from this study showed that the increase in SWS at high T_{amb} was abolished after destruction of WSN of the POA. This clearly indicates that WSN of the POA mediate the warm T_{amb} related increase in SWS. This supports several earlier reports that the neurons of the POA play a key role in coordinating sleep and T_b regulation (10, 19, 21, 22, 56–58). This further supports the concept that the basal forebrain and hypothalamus should be considered as part of the neural structure that integrate sleep with several homeostatic mechanisms including T_b regulation (12, 54, 59, 60–62).

The REM sleep curve was shifted to the right and the response was even exaggerated at 33°C after the destruction of WSN of the POA. This shows that the stimulus for the increase in REM sleep at higher than preferred T_{amb} is not provided by the WSN of the POA. Stimulation of non-preoptic WSN may be responsible for the increased REM sleep at 33°C. On the other hand, it also shows that the REM sleep generation is under some sort of inhibitory control of the WSN of the POA. Destruction of these WSN of the POA releases the T_{amb} related REM stimulating mechanism from its inhibitory control.

Earlier study had shown that both SWS and REM sleep were increased at 33°C when peripheral warm receptors were destroyed (27). This showed that the central WSN have an inherent tendency to increase SWS and REM sleep, but the inputs from peripheral warm receptors prevent the T_{amb} mediated sleep response from overshooting beyond 30°C. Peripheral warm receptors could be

influencing SWS by acting at the level of the POA. But they may have a direct influence on the REM generating mechanism in the brainstem, in addition to the above mentioned indirect influence through the POA.

The behavioral thermal preference selection was not altered after the destruction of WSN of the POA, as shown in the present study, and also after the destruction of peripheral warm receptors, as reported in the previous study (27). It has to be assumed that the extra preoptic WSN are sufficient and capable of eliciting homeostatic regulation of thermal preference selection, which is an important component of behavioral thermoregulation.

The hypothermia inducing effect of subcutaneously injected capsaicin (test dose) was reduced after intrapreoptic injection of capsaicin. It is reasonable to assume that the reduced hypothermia was due to the destruction of WSN of the POA (43). Electron microscopical investigation had shown ultra structural changes in one type of POA neurons even months after capsaicin treatment. It was claimed that these neurons have a warm sensor function (46).

In summary, the present observations and the earlier studies collectively suggests that the T_{amb} is an important determinant of both quantity and quality of sleep. T_{amb} exerts its influence through peripheral warm receptors and central thermo-sensitive neurons. WSN of the POA have an inherent tendency to increase SWS. WSN of the POA may have an inhibitory influence on REM sleep generating mechanism.

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