Japanese Journal of Physiology, **44**, 231–242, 1994

Changes in Sleep-Wakefulness after Kainic Acid Lesion of the Preoptic Area in Rats

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Abstract The role of the preoptic area (POA) neurons in the regulation of sleep-wakefulness (S-W) has been investigated in this study. The cell-specific neurotoxin, kainic acid (KA), was injected (0.8 μg in 0.2 μl) intracerebrally for lesioning of the POA. S-W was assessed (on the basis of EEG, EMG, and EOG recordings) for a day before bilateral lesion of the POA, and for 3 weeks after the lesion. There was an increase in wakefulness, and a decrease in all the stages of sleep after KA lesion of the POA. The reduction in deep slow wave sleep (S2) and REM sleep (PS) were more marked than light slow wave sleep (S1), and these had not shown any recovery even after 3 weeks of lesion. Two days after the lesion, the reduction in sleep was much more marked during the daytime than at night. There was an increase in locomotor activity, especially during the daytime, though it was only statistically significant on the 6th and the 10th day after the lesion. This study shows that the POA neurons are involved in the induction and maintenance of sleep. The lesion did not have a long lasting effect on the circadian distribution of sleep but the changes in locomotor activity seem to persist for a longer period.

Key words: preoptic area, kainic acid, sleep, wakefulness, locomotor activity.

The preoptic area (POA) is known to regulate slow wave sleep and REM sleep (PS) [1–3]. Nauta described it as the area for the "capacity of sleeping" on the basis of surgical destruction studies in rats [2]. Severe suppression of sleep was also reported after electrolytic lesion of the POA in rats [1] and cats [3]. Recently, radiofrequency lesions of the POA have shown that this area is important not only for sleep, but also for the regulation of the sleep-wakefulness (S-W) cycle [4]. The electrolytic and radiofrequency lesions destroy both the cells and fibers of passage. It could be argued that the changes in sleep after these lesions, might have resulted from the destruction of fibres as the POA has cells and fibers of passage [5]. This

Received on September 16, 1993; Accepted on April 18, 1994

argument holds true for the stimulation studies also. Low frequency stimulation of the basal forebrain/POA induced sleep, whereas high frequency stimulation induced wakefulness in freely moving cats [6–8]. On the other hand, alterations in S-W brought about by thermal [9] and chemical [10–12] manipulations of the POA suggest the involvement of neurons of this area in these changes. Studies to resolve this problem by lesioning the POA with cell-specific neurotoxin in cats have shown a marked suppression of sleep [13, 14], but the changes in day and night S-W, after neurotoxic lesion, assessed on the basis of 24-h recording, have not been reported so far. Reports on the changes in S-W, after the lesion of the POA cells in rats, are also lacking in the literature. The 24-h recording and analysis of day and night data are essential for the better understanding of the S-W cycle. To achieve these objectives, the POA of rats were lesioned with cell-specific neurotoxin, kainic acid (KA), and the changes in S-W were monitored for 3 weeks. The locomotor activity was also monitored simultaneously.

MATERIALS AND METHODS

The study was conducted on adult male Wistar rats weighing between 200 and 275 g. The rats were kept in a room with controlled temperature ($25 \pm 1^{\circ}C$) and light (14 h light : 10 h dark). The illumination during the light period was kept above 200 lx and that during dark period was maintained below 5 lx. Food and water were provided ad libitum. All the rats were screened for normal rest-activity cycle. Locomotor activity was monitored by a digital photo-actometer (Techno, India). Only those rats which showed a stable pattern of rest-activity cycle (30% more activity during night, as compared to light period), were used for this study. Under sodium pentobarbitone anesthesia (40 mg/kg bw), electrodes for EEG, EMG, and EOG recordings were chronically implanted. Bilateral screw electrodes were fixed onto the skull, above the frontal area (2 mm anterior to the bregma and 4 mm lateral to the mid-sagittal suture) for recording EEG [15]. EMG electrodes, consisting of flexible radiowire with a stainless steel loop attached to one end, were placed onto the dorsal cervical neck muscles. Similar electrodes were placed close to the external canthus of the eye for EOG recording [10, 16]. These electrodes were connected to an IC socket, which in turn was fixed on the skull with dental cement. The animals were then habituated to the recording cage (measuring $30 \times 30 \times 30$ cm), which was also maintained in lighting and temperature conditions identical to that of the animal house. Recovery from the surgical trauma was assessed from the observations of rectal temperature, food and water intake, behaviour, and activity. Most of the rats attained normal (pre-operative) levels of these parameters within 2 d of surgery. Rats were then trained to move around freely in the recording cage, with the attached cables. Reference data of S-W was recorded 4–7 d after surgery. Flexible cables with connectors, plugged to the rat's head (for recording EEG, EMG, and EOG), were taken out through a micro-swivel. These were fed to the input of a polygraph, along with the output of the

activity recorder. All the parameters for the assessment of S-W (EEG, EMG, EOG), as well as locomotor activity, were recorded continuously for 24 h (from 19:00 h to 19:00 h next day) in an 8-channel Grass 7 B polygraph (Grass Instrument Co., Quincy, USA), before lesioning the POA. The behavior of the rat was also observed throughout the experiment. The rats had access to food and water ad libitum throughout the recording session.

After completing the pre-lesion recording, the animals were anesthetized with sodium pentobarbitone (40 mg/kg bw), KA (Sigma, St. Louis, USA) at a dosage of 0.8 μg in 0.2 μl distilled water, neutralized with NaOH, was injected stereotaxically into the left POA at coordinates *A* 7.8, *H* −1.5, and *L* 0.6, as per De Groot atlas [17]. The same dose of KA was injected into the right POA, 48 h after the first injection. Our preliminary studies have shown that deep anesthesia (40–45 mg/kg bw) during the lesioning reduces the mortality rate. Two-stage lesioning was also resorted to reduce the mortality [18, 19]. KA was infused into the POA, at a rate of 0.1 μl/min, with the help of a slow injector (Palmer, England). The injector cannula was kept in place for 3–5 min to facilitate better diffusion of the drug. Of the 27 lesioned rats, 6 died within hours after the second injection of KA into the POA. Eight rats died after they recovered from anesthesia, but before the first post-lesion recording. Another seven did not survive until the end of the study, though a few post-lesion records were taken. The results presented in this report are based on the data of six rats which survived for 22 d after the lesion, as it was possible to complete the study (including histological examination) in these rats only. Parameters of assessment of S-W were monitored on the 2nd, 4th, 6th, 10th, 14th, 18th, and 22nd days after the second injection of KA, in addition to the pre-lesion record.

The 24 h S-W records were split into 30 s epochs, and visually scored [20, 21]. The wakeful period was classified into two stages, namely active wakefulness (W1), and quiet wakefulness (W2). The sleep period was classified into three stages, namely light slow wave sleep (S1), deep slow wave sleep (S2), and REM sleep (PS). EEG during W1 stage had low amplitude, high frequency (desynchronized) waves (Fig. 1). EMG and EOG record had gross body movement and eyeball movement artifacts, respectively. Locomotor activity recording, mostly coincided with movement artifacts. The animals showed grooming, scratching, and orienting activities during this period. During W2, EEG remained desynchronized. EMG, though remained high, did not show any movement artifacts. Spiky movements produced by the eyeballs were also practically absent. No locomotor activity was recorded, and the animals were found sitting quietly during this period. S1 stage was characterized by low frequency, high amplitude (synchronized) EEG spindle waves. There was considerable reduction in EMG activity, and an absence of locomotor activity. Rats assumed a sleeping posture during this period. S2 was characterized by continuous electrocortical slow wave activity, where synchronized waves were not seen as separate spindles. There was further reduction in EMG as compared to that during S1. The PS was characterized by desynchronized EEG,

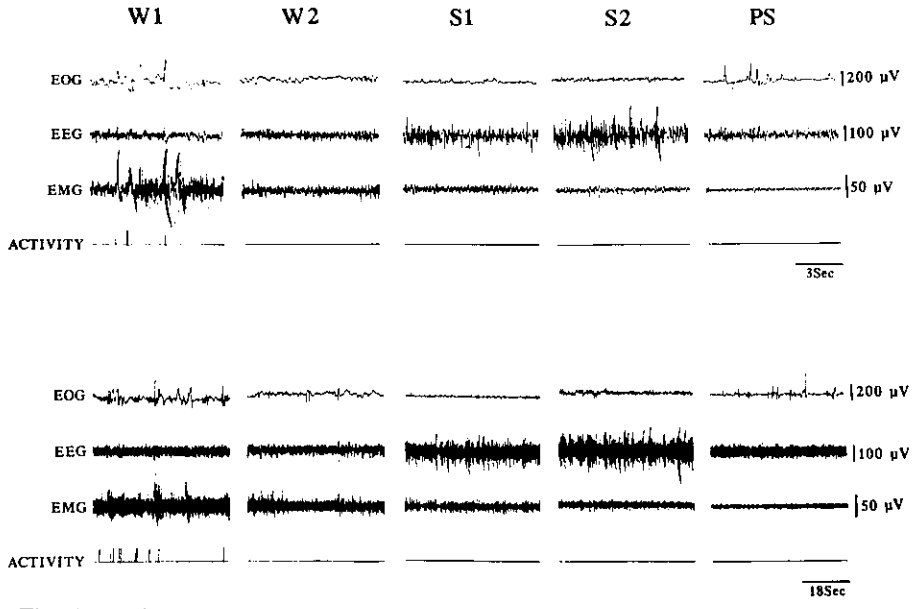

Fig. 1. Polygraphic recordings, at fast speed of 10 mm/s (upper tracings) and at slow speed 100 mm/min (lower tracing) of electro-oculogram (EOG), electroencephalogram (EEG), electromyogram (EMG), and locomotor activity of rat during different sleep-wakeful stages. W1, active wakefulness; W2, quiet wakefulness; S1, light slow wave sleep; S2, deep slow wave sleep; PS, paradoxical sleep.

drastic reduction in EMG and spiky waves in the EOG. Pre-lesion values of each of the different stages of S-W were compared with the post-lesion values of different days using Student's *t*-test. At the end of the experiment, the rats were anesthetized with sodium pentobarbitone (45 mg/kg bw) and perfused with 10% formalin. The brains were sectioned and stained with cresyl violet for histological examination of the lesion site.

RESULTS

The control (pre-lesion) record showed that the rats spent a greater part of the time (54%) in sleep. S1 and W1 occupied most of the sleep and wakeful periods respectively (Fig. 2). The reduction in sleep (which was evident 48 h after the POA lesion) reached a maximum on the 6th day (Fig. 3). Though there was a tendency towards recovery after that, the pre-lesion level of sleep was not attained even after 3 weeks. The increase in W1 was more significant on the 6th and the 22nd days, but the W2 was significantly increased only from the 2nd to the 10th day after the lesion. Though the reduction in S1 was significant throughout the period after the

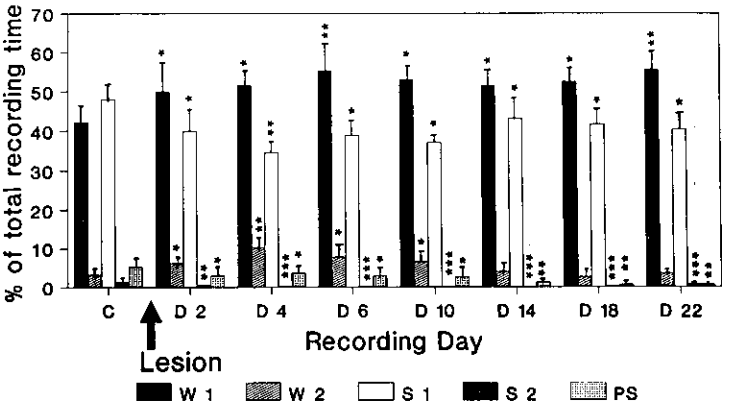

Fig. 2. Bar diagram shows the values (mean±SD) of all stages of sleep-wakefulness (W1, W2, S1, S2, PS) 2d (D2), 4d (D4), 6d (D6), 10d (D10), 14d (D14), 18d (D18), and 22d (D22) after the lesion of POA with KA. The post-lesion values of different stages are compared with pre-lesion control recordings (C) (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

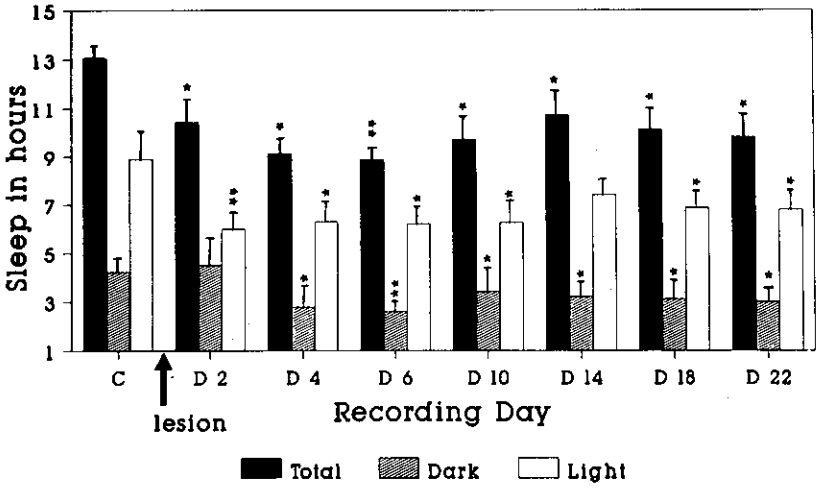

Fig. 3. Bar diagram shows the values (mean±SD) of total sleep (along with dark and light period sleep) after the POA lesion. The time spent in sleep is shown in Y axis. D2, D4, D6, D10, D14, D18, and D22 are the measurements of total sleep after 2, 4, 6, 10, 14, 18, and 22d, respectively, after the lesion of the POA. C, control record before lesion (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

lesion, the maximum reduction was noticed on the 4th day. S2 showed a highly significant reduction from the fourth day to the end of the study. A reduction in PS was also recorded after the 2nd day, but became more marked towards the 2nd

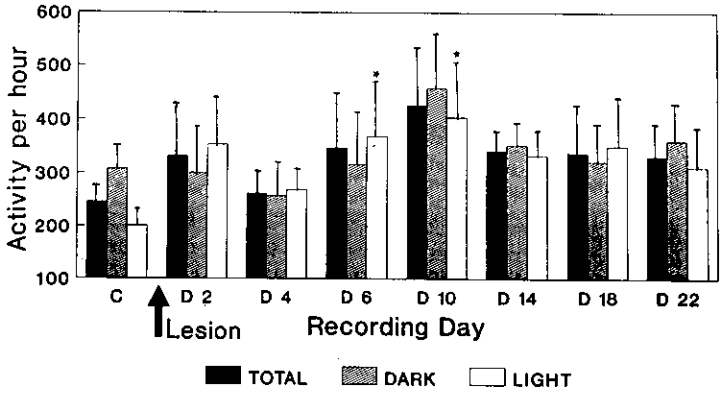

Fig. 4. Bar diagram shows the locomotor activity count (mean±SD) before (C) and 2, 4, 6, 10, 14, 18, and 22 d (D2, D4, D6, D10, D14, D18, and D22, respectively) after the POA lesion ($*p<0.05$).

week. When the light and dark period S-W were assessed separately, it was found that the animals spent 63.5% and 42.3% of the time in sleep during the light and dark periods, respectively, before the lesion (Fig. 3). Reduction in sleep, immediately after 48 h, was more marked during the light period than during the dark period. There was an increase in locomotor activity (though statistically not significant) after the POA lesion, which attained a peak on the 10th day (Fig. 4). When the light and dark period activities were assessed separately, the increase in light period activity was found to be significant on the 6th and 10th days after the lesion.

The lesion of the POA was confirmed by histological examination. KA injection produced lesions of varying sizes ranging from 0.5 to 1.5 mm in diameter (Fig. 5). At the lesion site, there was a marked loss of neurons. The surviving neurons were mostly hyperchromatic. Gliosis was observed at the lesion site (Fig. 6). The neuronal damage was maximum in the medial POA where KA was injected. The damage extended to the lateral POA also. It was difficult to demarcate the boundary of the lesioned site, as the area of neuronal destruction gradually merged with the intact adjoining regions. It was not possible to correlate the extent of the lesion with the severity of changes in S-W. It is important to note that the suprachiasmatic nuclei were intact in five rats. In one, this nucleus was partially damaged, but changes in S-W remained the same as in other rats with the POA lesion.

DISCUSSION

Local injection of KA-produced neuronal cell-selective lesion of the POA. A decrease in sleep and an increase in wakefulness were seen 2 d after the lesion, and

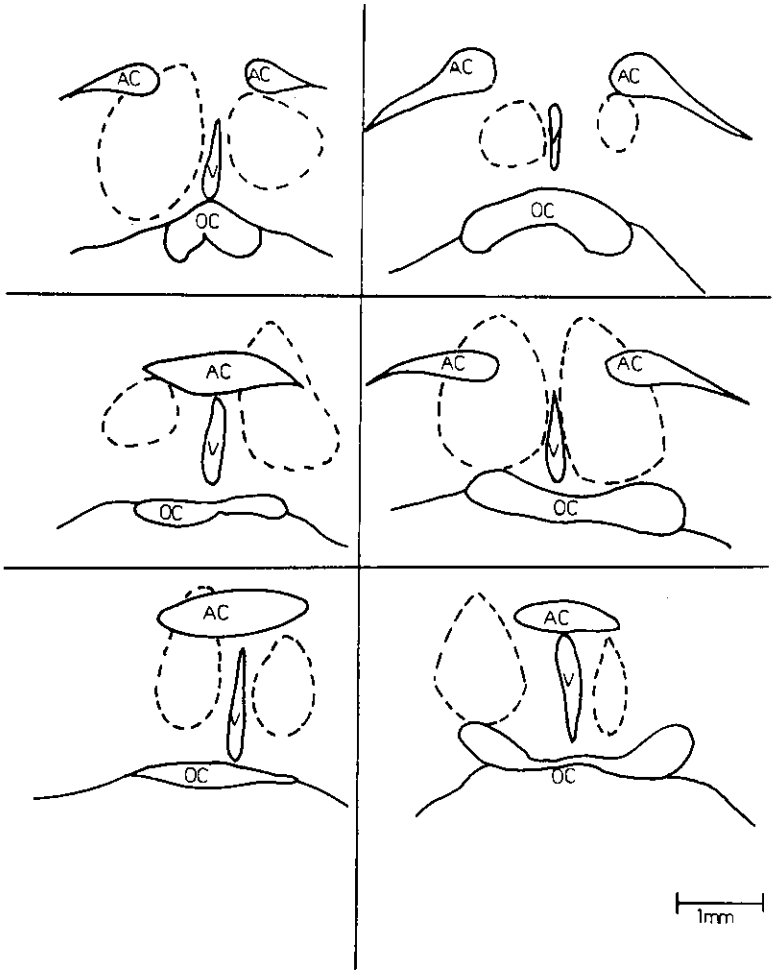

Fig. 5. Camera lucida drawing of coronal section (passing through POA) showing the KA lesioned area of six rats, which survived for three weeks. Dotted circles on either side of the 3rd ventricle (V) indicate the area of severe or moderate neuronal loss. AC, anterior commisure; OC, optic chiasma.

this alteration in S-W was maintained even after 3 weeks. The injection procedure per se could not have produced this change as earlier report from our laboratory demonstrated that no significant change in S-W was observed after 2 d of saline injection into the POA [21]. The different stages of S-W were not altered in an identical manner. Though the increase in W1 persisted throughout the study, the W2 duration attained the pre-lesion level after the 10th day. On the other hand, the decrease in all the stages of sleep (S1, S2, PS) persisted even 3 weeks after the lesion. The electrolytic lesion of the POA in cats showed a similar trend initially,

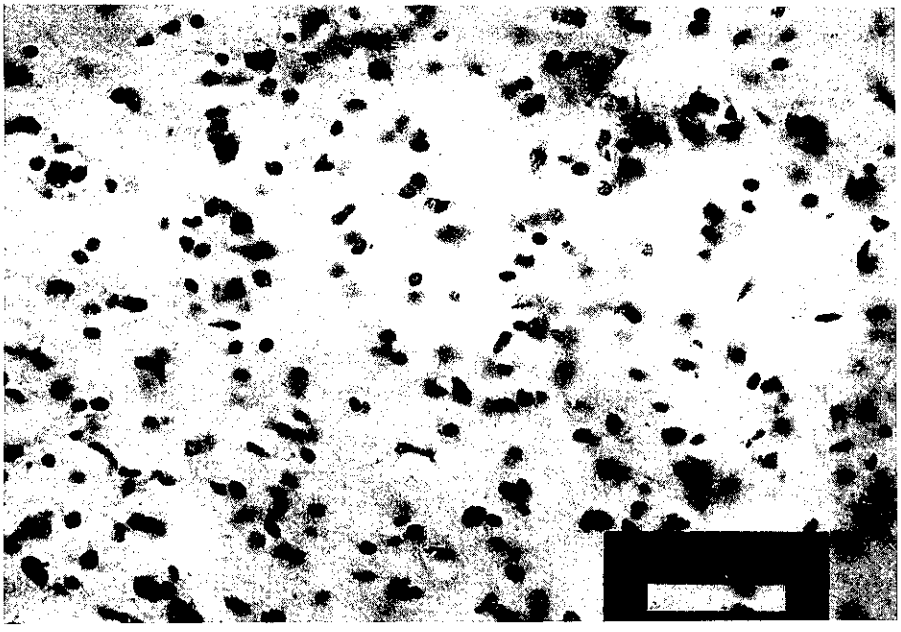

Fig. 6. Photomicrograph of the POA after KA injection, showing mostly glia. Bar : 50 μm.

but there was a recovery in sleep period by the 3rd week [3]. In the present study, S1 and S2 showed maximum reduction by the 4th day and PS by the 14th day. The S1 in rats, after KA lesion, did not show any recovery (except for the change from the 4th to 6th day) unlike in cats after electrolytic lesion [3]. The present observation is also not comparable to a study in cats, in which S1 was not much affected after lesioning with ibotenic acid, another neurotoxic agent [13], but they also observed a severe reduction in S2 and PS. However, according to one recent study, there was an increase in PS in rats after radiofrequency lesion of the medial POA [4]. As the different S-W stages were showing independent trends of change, it is clear that the influence of the POA on different stages is not identical.

The time course of increase in locomotor activity (though not significant) did not coincide with the changes in wakefulness. Still the possible impact of increased W1 on the locomotor activity cannot be ruled out as they both showed a peak at around 6 to 10 d after the lesion. Hyperactivity was also observed by other workers after the POA lesion [22–24], but the quantification of day-night rest-activity pattern after KA lesion of the POA has not yet been reported. Locomotor activity in rats is considered a good parameter for the assessment of the circadian rhythm [25]. So some of the increased daytime activity observed in this study in rats, with intact suprachiasmatic nuclei, suggests a possible role of the POA in circadian rhythm, in addition to its involvement in other important functions.

Though KA produces initial excitation of the neurons, it leads to their destruction by 24 h [26]. So, the suppression of sleep recorded from 48 h onwards after KA injection was the result of neuronal loss. The persistence of hyposomnia throughout the experiment (for 3 weeks after the lesion) further supported this conclusion. The high mortality rate soon after the second KA injection could be due to the enlarged area of stimulation and lesion, pulmonary edema, cardiovascular dysfunction, myocardial necrosis, gross hematuria, renal pathology, hyperthermia, and disturbance in thermoregulation as reported by previous investigators [19, 22, 23, 27–29].

The type of lesion of the POA seems to affect the changes produced in S-W. Though the trend of changes produced by the KA lesion was not much different from that caused by other types of lesions, the former failed to produce total sleeplessness in any of the rats. Surgical and electrolytic lesion, producing extensive destruction of the cells and fibers (and also adjoining areas), has produced total suppression of sleep and death within 3 to 10 d in some rats and cats [2, 3]. So it could be argued that the destruction of fibers in the POA is responsible for the drastic reduction in sleep in these animals, but the radiofrequency lesion of the medial POA in rats, which causes destruction of cells and fibers, also did not produce total abolition of sleep [4]. Instead, a reduction in sleep during the light period (daytime), a compensatory increase during the dark period, and recovery of total sleep deficits by 1 week were reported [4]. In contrast, there was suppression of sleep during day and night after KA lesion. A drastic reduction in daytime sleep (without change in nighttime sleep) was observed only on the 2nd day. Moreover, there was no recovery of total sleep period even after 3 weeks. More circumscribed lesion by radiofrequency current might not be responsible for the short-lasting S-W changes. Rats with smaller and larger lesion showed nearly identical reduction in all the stages of sleep in our study.

Insomnia may be due to the decreased activity of sleep inducing peptides [30]. It is known that the POA is one of the sites of action of sleep-inducing prostaglandin D2 and uridine [31–33]. The disappearance of this site of action may contribute to the suppression of sleep. The POA lesion may also result in the release of the waking centers like the posterior hypothalamus [34] and mesencephalon [35], from its inhibitory influence. Even though the POA lesion produces total abolition of male sex behavior [36] and severe disturbance in thermoregulation [14, 23, 24], it is not clear whether these functional deficits are linked to the changes in S-W function. Induction of sleep in cats by local warming of the POA shows that the regulation of sleep and thermoregulation by the POA are interdependent [1, 9, 37, 38]. A transient increase in sleep along with an increase in brain temperature was noticed even when the POA lesioned cats were exposed to a high ambient temperature [14]. This would suggest that the compensation of sleep by warm ambient temperature could take place even in the absence of POA.

The present study reveals that the POA neuronal population is important for the modulation and maintenance of sleep. The rats showed longer periods of

wakefulness and shorter periods of sleep after the destruction of POA neurons. Active wakefulness and deeper stages of sleep were more affected after the lesion.

Supported by the research grant No. 27/90-EMR-II of Council of Scientific and Industrial Research (CSIR), India. The help rendered by Dr. K. R. Sundaram, Department of Biostatistics, A.I.I.M.S., in the analysis of data is acknowledged.

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