REVIEW
Sleep is a reversible behavioral state of perceptual disengagement from and unresponsiveness to the environment. Based on a constellation of physiological parameters, two separate states have been defined within sleep: Non Rapid Eye Movement Sleep (NREM sleep) and Rapid Eye Movement Sleep (REM sleep).

The Non-REM sleep in humans is conventionally subdivided into four stages, which, though somewhat arbitrarily, are defined relatively and precisely, along one measurement axis, the Electroencephalogram (EEG). The EEG pattern in NREM sleep is commonly described as synchronous, with such characteristic waveforms as sleep spindles, K-complex and high-voltage slow wave sleep. The four NREM stages: stage-I, stage-II, stage-III and stage-IV in humans roughly parallel "depth of sleep" continuum, with arousal thresholds generally lowest in stage-I and highest in stage-IV sleep. NREM sleep is usually associated with a dearth of mental activity. Therefore, NREM sleep on the basis of brain status can be defined as **NREM sleep is relatively inactive, yet actively regulating brain in a movable body.** REM sleep, by contrast, is defined by EEG activation, muscle atonia and episodic bursts of rapid eye movements. The mental activity of human REM sleep is associated with dreaming based on vivid dream recall from approximate 80% of arousal from this state of sleep (Dement and Kleitman, 1957). Inhibition of spinal motor neurons via- brainstem mechanisms mediates suppression of postural motor tonus in REM sleep. Therefore, REM sleep on the basis of brain status can be defined as **REM sleep is a state of highly activated brain stage in a benumbed body.** The normal adult human enters into sleep through NREM sleep and REM sleep does not occur until 80 min of a bout of NREM sleep. Thereafter, NREM sleep and REM sleep alternate cyclically throughout the night with a period of about 90 min. The first cycle of sleep in the normal adult begins with stage-I sleep, which generally persists for only a few (1-7) minutes at the onset of sleep. Stage-II NREM sleep, signaled by sleep spindles or K-complexes in the EEG, follows brief episode of stage-I sleep and continues for about 10 to 25 min. In stage-II sleep, a more intense stimulus is required to produce an arousal. The same stimulus that produces arousal from stage-I sleep will often result in an evoked K-complex but no awakening in stage-II sleep (Carskadon and Dement, 1989).
1. CHARACTERISTIC FEATURES OF SLEEP IN THE NORMAL YOUNG HUMAN

- A normal young human enters sleep through NREM sleep.
- NREM and REM sleep alternate with a period near 90 min.
- Slow wave sleep predominates in the first third of night and is linked to the initiation of sleep.
- REM sleep predominates in the last third of the night and is linked to the circadian rhythm of body temperature.
- Stage-I sleep generally comprises about 2 to 5 percent of sleep.
- Stage-II sleep generally comprises about 45 to 55 percent of sleep.
- Stage-III sleep generally comprises 3 to 8 percent of sleep.
- Stage-IV sleep generally comprises 10 to 15 percent of sleep.
- REM sleep is usually 20 to 25 percent of sleep, occurring in four to six discrete episodes in a sleep period of about 8 hrs.

2. SLEEP AND HOMEOSTATIC FUNCTIONS

Mammalian sleep shares several properties with homeostatic behaviors such as feeding and drinking including relatively constant daily consumption, increased appetite during forced sleep deprivation, compensatory 'rebound' increase in consumption after deprivation is ended and at least in some species, death after about 2-3 weeks of complete deprivation (McGinty and Siegel, 1983; Rechtschaffen et al., 1983).

Sleep also incorporates thermoregulatory process as it has been observed that during sleep, heat loss processes e.g. reduced heat production, cutaneous vasodilation or sweating etc. are initiated. Heat loss during sleep is distinctive in certain ways that:
Sleep related cooling can be delayed and integrated with circadian rhythms.
- The reduced heat production is facilitated by the complete behavioral suppression associated with sleep.
- Brain cooling is augmented by coincident reduction of cerebral metabolic rate.

3. Correlation Between Sleep-Wakefulness and Thermoregulation

It has been hypothesized that NREM sleep is controlled by thermoregulatory mechanisms of the preoptic anterior hypothalamus. The evidences come into sight from the fact that how ardently these two mechanisms (sleep-wake and thermoregulatory mechanisms) are linked. The facts, which advocate the ardent link between sleep and temperature, include:

I. Accumulated heat load augments NREM sleep and
II. NREM sleep evokes heat loss processes, which decrease the heat load.

The hypotheses, which support the view that NREM sleep is controlled by the thermoregulatory mechanism, are based on the following observations.

[A] Augmentation of NREM sleep due to temperature:

Afternoon or evening hot bath immersion increases subsequent stage 3-4 sleep in humans (Horne and Reid, 1985; Bunnell et al., 1988). The increase in stage 3-4 sleep after intense exercise was found to be dependent upon body heating and could be eliminated by fan cooling during the exercise (Horne and Moore, 1985). Increase in stage 3-4 sleep in humans reflects increase in the intensity of sleep (Borbely et al., 1981). Heat loads also increase total subsequent NREM sleep in rats (Moraity et al., 1993).
NRJEM sleep dependent changes in brain and body temperature:

Brain and body temperature fall during sleep (Obal, 1984). These fall are regulated and are superimposed on the circadian temperature rhythm (Mills et al., 1978). Hypnogenesis integrates cerebral, behavioral, hormonal and autonomic effector elements to induce heat loss and reduce heat production (Parmeggiani, 1980). NREM sleep is characterized by nearly complete behavioral suppression and greatly reduced metabolic rate (Kennedy et al., 1982). These basic features of NREM sleep can be interpreted as thermoeffector mechanisms, facilitating a temperature drop by reducing heat production. Therefore, since heat load increases the intensity or duration of NREM sleep and sleep provides cooling, this defines a homeostatic system (McGinty and Szymusiak, 1990). Homeostatic features of sleep rhythm are generated by integration of heat load associated with waking; therefore, it is reasonable to believe that both the processes are linked. Simulations under entrained conditions show that the model closely mimics typical features of human sleep rhythms, including a biphasic daily pattern of sleepiness and sleep-onsets and awaking fixed in a descending and ascending phase of the temperature rhythm, respectively.

4. **FUNCTIONAL EFFECTS OF THERMOREGULATORY CONTROL OF SLEEP**

One of the functions of thermoregulatory control of sleep is periodical brain and body cooling. It has been suggested that sleep provides rest for certain cerebral processes (Horne, 1988). Long lasting cerebral activity leads to cerebral fatigue and lowering of cerebral metabolism in conjunction with SWS reverses the fatigue (McGinty and Szymusiak, 1990). Long-term sleep deprivation exhibits sustained hypothermia, reduced EEG amplitude and rapid death of rats (Rechtschaffen et al., 1983; Bergmann et al., 1989; Benca et al., 1989). These sleep-deprived rats exhibited a striking change in heat retention mechanisms. Several studies have shown that sleep-deprived humans also exhibit lower daytime body temperatures than controls (Froberg, 1977). These findings suggest that the elevated waking
temperatures in homeotherms can not be sustained without periodic cooling during sleep. Maximal psychomotor (Aschoff et al., 1972) and aerobic (Bennett and Ruben, 1979) performance are associated with peaks in circadian temperature rhythm and subjective alertness is positively correlated with temperature across the circadian cycle (Czeisler et al., 1980). The cool human brain is experienced as groggy in order to be alert, therefore our brain must be warm but not too warm. The homeothermic brain is hitherto sensitive to hyperthermia, as brain temperature slightly above normal, is associated with disorder of mental status. Loss of consciousness is the initial effect of heat stroke, which usually causes irreversible neuropathology and furthermore sleep deprivation is thought to increase susceptibility to heat stroke (Shibolet et al., 1976). A functional role of sleep in immune mechanisms has been proposed by Krueger and colleagues as a result of the observations that many immune factors (muramyl peptides, IL-1, tumor necrosis factor, interferon-α2 etc.) promote sleep and simultaneously most of those factors are pyrogenic as well (Krueger and Majde, 1994). However, sleep can be enhanced during the plateau phase of a fever (Kent et al., 1988). There may be additive effects of sleep and fever in support of immune defense mechanisms. Therefore, possible functional effects of thermoregulatory control of sleep can be:

- Energy conservation.
- Reduced cerebral metabolism to restore certain fatigable cerebral process.
- Regulation of mean temperature below waking levels to avoid biophysical disorders resulting from sustained high waking temperatures.
- Facilitation of immune response.

It is possible that a complex process like sleep could serve each of the above functions, with the functions having somewhat different importance in different species.
5. **SLEEP-GENERATING SYSTEM**

Following the acceptance in the 1940s and 1950s of the existence of an activating system, many physiologists believed that sleep was the result of fatigue and decrease in activity of this system, thus representing a passive deactivation (Moruzzi, 1972). However, in experimental studies employing transection of the brainstem, it was found that sleep could be diminished so as to indicate that active sleep-inducing structures must be present in the brain. Moruzzi and his colleagues were thus to show that transection of the brainstem behind the oral pontine tegmentum resulted in a total insomnia (Batini et al., 1958). These results indicated that sleep-generating structures were located in the lower brainstem. Low frequency stimulation of medullary reticular formation within the medulla, particularly the dorsal reticular formation and the solitary tract nucleus, was shown to produce cortical synchronization indicative of slow wave sleep in an awake animal preparation (Magnes et al., 1961). Conversely, lesions of the dorsal reticular formation and the nucleus of the solitary tract produced desynchronization of the EEG in sleeping animal preparation (Bonvallet and Allen, 1963). All these results suggested that neurons, in the dorsal medullary reticular formation and the nucleus of the solitary tract could generate sleep. The mechanism was hypothesized to involve inhibition of the rostrally located neurons of the ascending activating system, although a direct synchronogenic influence upon forebrain system was also considered a possibility.

Original studies of Bremer, in 1965, utilizing the *cerveau isole* preparation had shown that synchronogenic structures must also be located in the forebrain. Cortical synchrony was continuous in such preparations, in which the brainstem influence was removed. It was discovered that synchronous cortical activity could be driven or recruited by low frequency electrical stimulation of midline thalamus (Morison and Dempsey, 1942). Hess and his colleagues subsequently showed that this stimulation also led to behavioral and EEG sleep in the animal with chronically indwelling electrodes, thus leading Hess to suggest that the *thalamus is the head ganglion of sleep* (Akert et al., 1952). However, lesion studies showed that
although the thalamus is necessary for the production of cortical spindles, it is not necessary for the generation of cortical slow waves and behavioral sleep, which persist following its complete ablation (Villablanca and Salinas-Zeballos, 1972).

In humans, von Economo in 1931 had noted that in certain cases of “encephalitica lethargica” in which insomnia was the prominent symptom, lesions were centered in the anterior hypothalamus. He thus postulated that a sleep facilitatory center was located in the anterior hypothalamus. This sleep center was conceived to be in opposition to and normally in balance with the sleep inhibitory or waking center of the posterior hypothalamus. Nauta later experimentally confirmed the existence of a sleep facilitatory region in the anterior hypothalamus and preoptic area by knife cuts (Nauta, 1946). Hess demonstrated that electrical stimulation of this area could elicit behavioral suppression (Hess, 1954). Neurons in this anterior region were postulated to exert an inhibitory influence upon the neurons of the ascending reticular activating system.

In the 1960s, Sterman and Clemente showed that electrical stimulation of the preoptic area and basal forebrain (including the nucleus of the diagonal band) led to drowsiness and behavioral and EEG sleep (Sterman and Clemente, 1962a; 1962b). Conversely, they found that lesion of basal forebrain, including the preoptic area, substantia innominata and nucleus of horizontal limb of the diagonal band, led to elimination or a decrease in sleep and a disruption of the sleep cycle (McGinty and Sterman, 1968; Lucas and Sterman, 1975). Villablanca and his colleagues found that animals without neocortex and striatum, thus called “diencephalic” cats and with sleep-inducing structures of both the lower brainstem and the anterior diencephalon largely intact did not show a normal sleep cycle, but instead a large decrease in sleeping time (Villablanca and Marcus, 1972). Electrical stimulation of both the caudate nucleus and the orbitofrontal cortex had been shown to produce cortical synchronization and behavioral sleep (Penaloza-Rojas et al., 1964; Liles and Davis, 1969a). Bilateral lesions of frontal cortex resulted in a permanent moderate reduction in sleep, whereas lesions of the caudate nuclei led to temporary decrease in sleep (Penaloza-Rojas et al., 1964; Liles and Davis, 1969b).
From early neuroanatomical studies, neurons in the hypothalamus and preoptic area have been known to provide an innervation to limbic forebrain structures including notably the septum, amygdala and frontal cortex and also send descending projections to the limbic midbrain region, in what Nauta termed the “limbic forebrain-midbrain circuit” (Nauta and Haymaker, 1969). Such descending projections would allow for a certain degree of modulation of midbrain reticular formation, although the main projection is actually focused more medially onto the paramedian tegmentum, central gray and raphe nuclei (Nauta and Haymaker, 1969; Swanson, 1976; Swanson and Cowan, 1979). Neurons in the hypothalamus as well as the bed nucleus of the stria terminalis and amygdala also project directly to the solitary tract nucleus and the adjacent tegmentum in the medulla, sending fibers through as well as to the parabrachial region in the pons (Ricardo, 1983). It has also been shown that the frontal cortex also projects directly to the solitary tract nucleus and the region, in addition to supplying an important input to the preoptic area of anterior hypothalamus and to the pontine parabrachial nuclei (Nauta and Haymaker, 1969; van der KD et al., 1984). These forebrain and lower brainstem structures thus appear to form by their inter-connections a complex regulatory system which seems to be involved in the sleep generation. Recently a direct cholinergic projections form neurons in the basal forebrain to the cerebral cortex, originating from neurons in the substantia innominata, nuclei of the diagonal band, septum and magnocellular nuclei have been proposed to be involved in inducing cerebral activity (Szymusiak, 1995).

Hence, the importance of the anterior hypothalamus, preoptic area and basal forebrain, in addition to the lower brainstem reticular formation, in the generation of sleep appeared fairly clear.

6. THERMOREGULATORY MACHINERY IN THE CENTRAL NERVOUS SYSTEM

The temperature of the inside of the body – the “core” – remains almost constant, within ± 0.6 °C day in and day out except when a person develops a
febrile illness. The average normal body temperature is generally considered to be 37 °C (98.6°F). Indeed, the nude person can be exposed to temperatures as low as 13 °C or as high as 60 °C in dry air and still maintain an almost constant internal body temperature. Therefore, it is obvious that the mechanisms for control of body temperature represent a beautifully designed control system.

The temperature of an object is a measure of the kinetic activity of its molecules, and is proportional to the amount of heat stored in the object. Therefore, body temperature is directly proportional to the heat in the body. On an average, the body temperature increases by 1 °C for each 0.83 Calorie stored per kilogram of body weight. In other words, the specific heat of the tissue is 0.83 Cal/kg/°C. For a 70 kg man, approximately 58 Calories of heat must be added to the body to raise the body temperature by 1 °C or 1.8 °F.

Heat is continually being produced in the body as a by-product of metabolism, and body heat is also continually being lost to the surroundings. When the rate of heat production is equal to the rate of loss, the person is said to be in a state of Heat Balance. But when the two are out of equilibrium, the body heat and the body temperature, will obviously be either increasing or decreasing.

1. Basal metabolism
2. Muscular activity (Shivering)
3. Thyroxin effect on cells
4. Sympathetic effect on cells
5. Temperature effect on cells

1. Radiation
2. Conduction
3. Convection
4. Evaporation

Balance of heat gain versus heat loss

Fig. 1.1
Regulation of Body Temperature

Instant regulation of body temperature is regulated by nervous feedback. It is known that medial preoptico-anterior hypothalamic area is an important thermoregulatory center in the brain. A variety of thermoregulatory responses can be elicited by changing the temperature of preoptic area of anterior hypothalamus (POAH). POAH warming evokes heat loss responses (Boulant, 1980; Boulant, 1991) and POAH cooling evokes heat production responses (Hellstrom and Hamel, 1967; Boulant, 1991). If POAH temperature is changed slightly above or below normal, there are changes in heat retention responses such as skin blood flow and thermoregulatory behavior.

7. PREOPTICO-ANTERIOR HYPOTHALMIC AREA: REGULATION OF SLEEP AND BODY TEMPERATURE

The term preoptic area (POA) was used for the first time by Herrick and Edinger. POA is situated bilaterally rostral to the hypothalamus along the supraoptic recess of the third ventricle (3V). Rostro-ventrally it begins at the level of the diagonal band of broca (DBB). Dorsally it extends up to the anterior commissure (ac) and its bed nucleus. Caudally its dorsal portion overlaps the anterior portion of the anterior hypothalamic (AH) area and laterally it merges with lateral hypothalamic area (LHA) at the level of dorsal to optic chiasma (OX). Medially, it is bordered by the periventricular nucleus (PVN) and 3V.

The history of involvement of POAH in sleep-wakefulness (S-W) dates back to the anatomo-clinical observations by von Economo and electrical stimulation study by Hess during early 20th century. The transection study by Nauta (1946) in rat set the stage for the multitude of works in this direction. Lesion, stimulation and single unit studies have demonstrated the role of POAH in sleep, wakefulness and the corresponding changes in the EEG (Nauta, 1946; Sterman and Clemente, 1962a, 1962b); McGinty and Sterman, 1968; Roberts and Robinson, 1969; Moruzzi, 1972; Lucas and Sterman, 1975; Kaitin, 1984; Velluti and Hernandez Peon, 1963; Mohan Kumar et al., 1984; Szymusiak and McGinty,
1986a, 1986b; Alam and Mallick, 1990; Benedek et al., 1979a, 1979b; Mallick et al., 1983). However, based on substantial literature, the POAH has been advocated to be predominantly a hypnogenic area. On the other hand lesion, stimulation as well as single neuronal studies have also demonstrated the role of POAH in the regulation of body temperature (Szymusiak et al., 1985; Boulant, 1991).

7.1 POAH AND SLEEP

The anatomical observation of von Economo (1931) on cases of sleeping sickness was the first to call the attention of sleep physiologist to the hypothalamic involvement in S-W. Studying the epidemic of "encephalitis" which had spread through the city of Vienna following the 1st world war, von Economo described several cases in which insomnia and chorea were the primary symptoms. In all those cases, inflammation was associated with the rostral hypothalamus, tuberal region and adjacent region of the striatum. Therefore, he concluded that rostral hypothalamus is a "Schlafsteuerungszentrum" or "sleep center". Subsequently, several studies were made to find out the involvement of brainstem and diencephalic structures in S-W and synchronization and desynchronization.

7.1.1 Transection studies:

It was Nauta (1946), who set forth to put the earlier observation of von Economo to an experimental test in rats. He made transections across the base of the brain at various frontal planes and observed that:

i) the chronic transections made in the rostral part of the hypothalamus, particularly at the suprachiasmatic level, made the rat alert, irritable and insomniac,

ii) whereas transections placed in the posterior hypothalamus produced persistent somnolence.
From these observations he concluded that the rostral half of the hypothalamus (conforming to suprachiasmatic and preoptic areas) is the site of specific importance for the capacity of "sleeping" whereas the posterior hypothalamus constitutes the "waking center". He further suggested that sleep might be caused by inhibition of the waking center (posterior and lateral hypothalamic area) by the sleep center. In a recent transection study, Szymusiak and McGinty (1990) recorded SWS in *cerveau isole* (complete transection of caudal midbrain, which disconnected thermoregulatory effectors from hypothalamic regulatory systems) cats at two ambient temperatures viz., 37.5 °C and 39.5 °C maintained by a thermostat. Sleep percentage was more than double during the warmer condition. They suggested that sleep is probably driven by temperature.

7.1.2 Lesion studies:

(a) Electrolytic lesion studies

Obrador (1943) showed that bilateral total electrolytic destruction of the hypothalamus and basal forebrain in cats led to total abolition of cortical synchronization. Sterman et al. (1964) reported that electrolytic lesion of the medial basal forebrain regions in the cats produced significant increase in wakefulness and EEG desynchronization, associated with reciprocal decrease in drowsy state. Lesions of rostral part of preoptic region and in the basal portion of the diagonal band of broca was also reported to induce a significant increase in wakefulness and decrease in sleep (Madoz and Reinoso - Suarez, 1968). McGinty and Sterman (1968), after a massive electrolytic lesion of preoptico-basal forebrain area (including AH area and basal forebrain) of cats reported a gradual and significant decrease in the sleep states, beginning approximately three days after surgery, becoming maximal (including cases of complete insomnia) after 1-3 weeks and lasting for 4-8 weeks. In addition, other physiological disturbances viz. loss of temperature regulation, prolonged aphagia and death from exhaustion were also marked. Lucas and Sterman (1975) attempted to study the effect of lesion of a relatively smaller area involving rostral portion of the suprachiasmatic nucleus and

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its immediate adjacent preoptic region in cats. They reported suppression of sleep and loss of normal sleep-wake cycle, followed by a recovery in which the mean cycle length remained shorter. However, no accompanying sign of other physiological disturbances viz. adipsia, prolonged aphagia and death from exhaustion, as reported by McGinty and Sterman (1968) were observed. Szymusiak and Satinoff (1984) recorded S-W at different ambient temperatures viz. 29°C, 25°C and 30°C before and after electrolytic lesion of mPOAH. The rats were insomniac at all ambient temperatures (Ta) for 1-2-post lesion days and after that sleep was highly dependent on Ta.

The ventrolateral preoptic nucleus (VLPO) in rats shows c-fos activation after prolonged sleep and the neurons here are considered to be sleep related. These cells provide GABAergic innervation to the major monoaminergic arousal system, suggesting that they may be a necessary part of the brain circuitry that produces sleep. The effects on sleep behavior in rats of cell-specific damage to the VLPO by microinjection of ibotenic acid were examined. It was observed that lesions of the central cell cluster of the VLPO (80-90% cell loss bilaterally) caused a 60-70% decrease in delta power and a 50-60% significant decrease in NREM sleep time (Lu et al., 2000). However, lesions medial or dorsal to the central cell cluster of the VLPO, where scattered VLPO neurons lie, causes small change in NREM sleep time but causes significant loss of REM sleep. The insomnia caused by bilateral VLPO lesions persisted for at least three weeks and during these periods there was no effect on the mean body temperature or its circadian variation after small lesions of the VLPO. These experiments showed that there are distinct preoptic sites which have primary effects on the regulation of NREM sleep, REM sleep and body temperature respectively (Lu et al., 2000).

(b) Chemical lesion studies

Szymusiak and McGinty (1986a) reported that neurotoxin (Kainic acid) induced lesion of the dorsal band of broca (DBB), lateral preoptic area (IPOA) and substantia innominata (SI) in cats suppressed sleep, particularly SWS and REM sleep. Sallanon et al. (1989) reported a long lasting insomnia after neurotoxic
lesions of the POAH. However, microinjection of GABA-ergic agonist muscimol into the rostral midbrain / posterior hypothalamus resulted in acute restoration of sleep. Szymusiak et al. (1991) reported that POAH (particularly consisting mPAOH) cell loss produced by N-methyl-DL- aspartic acid caused reduction of SWS and REM sleep for 7 and 5 weeks respectively at 23°C (the temperature at which cats were acclimatized with), and at 33°C sleep was nearly twice to that at 23°C, and almost restored to prelesion values. This report suggests that SWS is probably driven by elevated brain temperature.

A precise and reversible inactivation of medial and lateral preoptic area by a local anaesthetic-marcain (Alam and Mallick, 1990) suggests that mPOAH is predominantly involved in sleep and IPOAH is more involved in wakefulness. Recently a lesion study showed that the loss of ventrolateral preoptic area's neurons produced profound insomnia but did not alter body temperature or its circadian pattern (Lu et al., 2000).

7.1.3 Stimulation studies:
(a) Electrical stimulation studies

Hess first developed a method of direct electrical stimulation of brain by surgical placement of electrodes. He reported that low frequency (8 pulse/sec) and low voltage stimulation applied to several forebrain sites including POAH produced drowsiness and sleep like behavior in free moving cats. Sterman and Clemente (1962a) reported that unilateral low rate stimulation of lateral preoptic region and adjacent diagonal band of broca in cat immobilized with gallamine resulted into EEG synchronization. The response was time locked with stimulation. The same authors, reported subsequently (1962b) that bilateral stimulation (with frequency 6.5-15 Hz.) of the same forebrain region, this time in unanaesthetized freely moving cats, was followed by appearance of behavioral sleep and EEG synchronization with very short delays. Hernandez-Peon (1962) reported that stimulation of IPOAH at frequencies 1-25 Hz induced drowsiness and sleep since the stimulation of the same area at higher frequencies (more than 25 Hz) induced cortical synchronization. Yamaguchi et al. (1963) reported that low frequency
stimulation (5-7 Hz) of the POAH induced sleep and the high frequency stimulation (200-300 Hz) of the same area induced wakefulness in cat. Sleep induction after low as well as high frequency stimulation of basal forebrain region has also been reported by other workers in cats (Nielson and Davis, 1966; Sterman and Fairchild, 1966). Further, Benedek et al. (1979a, 1979b) reported that high frequency stimulation of the area which induced synchronization on low frequency stimulation viz. the mPOAH, induced desynchronization of cortical EEG. Behavioral and EEG pattern of sleep have also been reported on electrical stimulation of preoptic and anterior hypothalamic area in squirrel and monkey (Perachio, 1969).

(b) Chemical stimulation studies

The very nature of sleep along with convincing evidences for a chemical basis of synaptic transmission led the biologists to investigate for a chemical and humoral mediator in regulation of sleep. It was attempted to mimic the release of possible chemical mediators by ip, icv, or local application into specific brain structures of naturally occurring classical or putative neurotransmitters.

(i) Cholinergic mechanism:

Application of micro quantity of acetylcholine (ACh) into upper mPOAH in cats induced sleep state (Hernandez-Peon et al., 1963; Velluti and Hernandez-Peon, 1963; Yamaguchi et al., 1964). Velluti and Hernandez-Peon (1963) further showed the action of ACh on sleep-wakefulness in the mPOAH, where application of atropine, an ACh blocker, induced alertness. The specificity of the area (upper mPOAH) was further confirmed by the application of ACh after electrolytic lesion of the same area, which did not lead to sleep (Hernandez-Peon et al., 1963). Sleep has been reported on application of eserine, an antagonist of cholinesterase, into the same site (Velluti and Hernandez-Peon, 1963). Moreover, carbachol (cholinergic agonist) administration into the POAH has also been reported to induce arousal (Yamaguchi et al., 1964) and "effective-defense" mechanism (Macphail and Miller, 1968). Recently it has been further shown by many groups
that carbachol administration into this area induces wakefulness (Baghdoyan et al., 1993; Imeri et al., 1995; Mallick and Joseph, 1997).

However there are contradictory reports stating the effects of ACh in the mPOAH on sleep-wakefulness; where as the primary consideration for the discrepancies between the results may be related to different animal species used in the study, the secondary consideration could also be the mode of application i.e., ACh crystals versus the microinjection. Although mPOAH area is devoid of cholinergic neurons (Gritti et al., 1993) but it seems to be an important cholinceptive zone to regulate sleep-wakefulness.

(ii) Serotonergic mechanism:
Presence of 5-HT (5-hydroxytryptophan) has been reported in the mPOAH by histofluorescence (Fuxe, 1965), biochemical (Saavedra et al., 1974), autoradiographic (Parent et al., 1981) and immunohistochemical (Simerly et al., 1984a, 1984b) studies. Application of 5HT in the POAH has been reported to induce sleep (Marczynski and Yamaguchi, 1963; Yamaguchi et al., 1964).

(iii) GABAergic mechanism:
GABA is a major inhibitory neurotransmitter in the mammalian central nervous system and its depressant actions are predominantly mediated by GABA<sub>A</sub> receptors (Decavel and Van den Pol, 1990). To understand the critical role of GABAergic system of basal forebrain, picrotoxin, an inhibitor of endogenous GABA, was injected into the substantia innominata / lateral preoptic area by Mogenson and Nielsen, 1983 and further by Swerdlow and Koob, 1987. They found that GABAergic system in the substantia innominata / lateral preoptic area regulates locomotor activity. To assess the involvement of GABAergic system in the regulation of sleep it has been emphasized that the hypnotic properties are mostly shared by agonistic modulators and by the selective agonists of the GABA<sub>A</sub> receptor complex. It has been observed in humans that a single oral dose of Gaboxadol (THIP), a partial GABA-A receptor agonist, was found to significantly increase sleep (Faulhaber et al., 1997). GABA concentration varies across sleep-wake
cycle in the different areas of brain. GABA concentration decreases in the posterior hypothalamus during REM sleep (Nitz and Siegel, 1996) and in the septal region of basal forebrain during wakefulness in freely moving normally behaving cats (Mallick et al., 1997). In both rats and normal sleeping individuals, GABA agonists are able to reduce sleep latency, increase sleep continuity and promote NREM sleep as well as occurrence of spindles (Lancel, 1999). Furthermore, it has been reported that GABA in the mPOAH by acting through its GABA_A receptors modulates NREM as well as REM sleep (Ali et al., 1999). All these observations indicate that GABAergic machinery in the basal forebrain including mPOAH plays an important role in the regulation of sleep-wakefulness. The presence of sleep related neurons in the basal forebrain were known since long but their neurotransmitter contents were not known. Recently it has been reported that sleep related neurons in the ventrolateral preoptic area are multipolar, triangular in shape and GABAergic in nature (Gallopin et al., 2000). They further showed that these neurons are inhibited by the neurotransmitters of wakefulness such as norepinephrine and acetylcholine. They also showed that most of these neurons are inhibited by serotonin but unaffected by histamine. Therefore, it seems that the reciprocal inhibitory interaction of such neurons with the norepinephrinergic, serotonergic and cholinergic waking systems is a key factor for promoting sleep.

(iv) Noradrenergic mechanism:

Noradrenaline (NA) was detected and proposed as a neurotransmitter in mammalian CNS about 38 years ago (Vogt, 1954). In the later years a significant contribution was made by Jouvet proposed the monoaminergic theory (1972) stressing the role of NA in the regulation of S-W. This model provided impetus for a great deal of research, directed at elucidating the noradrenergic basis of the mammalian S-W regulation. Electrolytic and neurotoxic lesions of catecholaminergic systems in the CNS as well as inhibition of catecholamine synthesis was followed by induction of sleep and decrease in waking activity (Monti, 1982). Locus coeruleus (L.C) area in the brainstem is a major source of norepinephrine because majority of the neurons in this area are noradrenergic
The single neuronal activity of LC showed that cells of this area have maximum firing rates during wakefulness, slow firings during NREM sleep and very low or totally abolished during REM sleep (Aston-Jones and Bloom, 1981). It has been observed that continuous electrical stimulation of locus coeruleus induced active wakefulness and reduced sleep particularly REM sleep (Singh and Mallick, 1996).

In the last two decades a number of experiments, primarily with i.p. or systemic administration of various agonists and antagonists of alpha-1, alpha-2 and beta adrenoceptors have been conducted to find out their involvement in the regulation of different phases of S-W (Putkonen and Leppavuori, 1977; Mendelson et al., 1980; Gillard, 1982; Stenberg and Hilakivi, 1985; Depoortere, 1985; Adrien et al., 1985).

Preoptic Noradrenergic System and Sleep-Wakefulness

There are considerable evidences, mostly through independent studies, that NE plays an important role in the POAH mediated changes in S-W. Isolated studies have been conducted to investigate the mechanism of action of NE on S-W by systemic or local injection of NE or its agonist and antagonist independently. The injection of NE into POAH induced wakefulness and alerting responses in cats (Hernandez-Peon, 1962; Hernandez-Peon et al., 1963; Yamaguchi et al., 1963). Similar results have been reported after NE injection into POAH in rats (Poole and Stephenson, 1979; Day et al., 1979; Tsoucaris-Kupfer and Schmitt, 1972; Mohan Kumar et al., 1984). However, Mohan Kumar et al. (1984) studied the effects of NE and phenoxybenzamine (PBZ), an alpha adrenoceptor blocker, into POAH on S-W of freely moving rats both during day and night. They reported that NE induced increase in wakefulness during day, whereas PBZ induced sleep at night. This led to the suggestion that alpha adrenoceptors in the mPOAH is probably involved in the wakefulness mechanism. The immunohistochemistry study of the catecholamine synthesizing enzymes, tyrosine hydroxylase (TH) and dopamine-beta-hydroxylase (DBH), in sections of POAH from perfusion-fixed male rats showed that TH is present in neuronal perikarya and processes in the anteroventral
periventricular region of the mPOAH, while DBH was only seen in the fibers and terminals (Reuss et al., 1999).

It is well accepted that the elucidation of central neuronal mechanism on the basis of peripheral or systemic drug administration is difficult due to their effects on the peripheral tissues. Therefore a better solution to this problem is local application of the chemical into specific brain sites. The studies related to the role of specific adrenoceptors in the POAH involved in S-W, on the one hand are limited while on the other hand some of the studies have reported diverse and sometimes contradictory responses. Some of the available studies suggesting the involvement of POAH adrenoceptors in S-W are illustrated as follows.

Tsoucaris-Kupfer and Schmitt (1972) reported that clonidine (CL), a powerful alpha-2 agonist induced sedation after injection into rostral hypothalamus including POAH. Mohan Kumar et al., (1986), investigated the effect of NE, its alpha adrenoceptors blocker PBZ and beta adrenoceptors blocker propranolol (PRO), on S-W after their independent injections into the POAH. It was reported that NE induced wakefulness, PBZ induced sleep whereas PRO was ineffective in influencing S-W. It was suggested that alpha adrenoceptors in the POAH are most likely involved in arousal mechanism. De Sarro et al., (1987) reported that injection of CL into 3rd ventricle produced behavioral sedation and sleep, whereas the injection of prazosin (PZ) as well as methoxamine (ME) was ineffective in inducing any significant change in S-W. The intraperitoneal as well as oral administration of α adrenoreceptor blockers significantly shortened quiet waking and prolonged slow wave sleep (Makela and Hilakivi, 1986; Kleinlogel, 1989). Furthermore, it has been reported that unilateral infusion of a β-agonist isoproterenol (ISO) into medial septum in forebrain elicited robust bilateral activation of EEG while β-antagonist timolol decreased EEG indices of arousal in urethane anaesthetized rats (Beridge et al., 1996).

From the results of these studies of injection of either agonist or antagonist of adrenoceptors, it was attempted to suggest that possibly all the adrenoceptors are involved in the regulation of S-W. In such experimental designs where an adrenoceptor agonist or antagonist is locally injected, more NE (than normally
present in the system) becomes available for other adrenoreceptors in the system and will keep them active. Therefore, it will be hard to establish the precise role of subtypes of adrenoreceptors. At that moment, the injection of different NE-ergic agonists and antagonists in combination, into the POAH simultaneously or one after the other was obligatory. The sequential injection of NE-ergic antagonist and agonist will keep one type of receptors inactivated while activating other adrenoreceptors. Therefore, the combination studies were necessary to understand the precise critical role played by each individual adrenoreceptor. For the first time Mallick and Alam (1992) did a study of its own kind on these lines. They reported that the injection of α-2 agonist (clonidine) into the POAH induced sleep while the injection of β agonist (isoproterenol) induced wakefulness. When both were given in a combination, the latter was relatively more potent. Blocking of α-1 and α-2 adrenoceptors primarily induced wakefulness. Clonidine induced sleep in the presence of both α-1 agonist or antagonist but if β- agonist (isoproterenol) was present in the POAH, the latter's effect dominated. By applying injections in several combinations, they hypothesized that NE by acting through α-2, β and α-1 adrenoreceptors in the mPOAH modulates sleep, wakefulness and body temperature respectively. Subsequently, Osaka and Matsumura (1995), by using glass electrodes and pressure injection techniques, have shown that α-2 adrenoreceptors inhibit sleep related neurons in POAH.

(c) Thermal stimulation studies

The local thermoceptive elements of POAH have been reported to induce EEG and behavioral changes with thermal stimulation of the POAH and adjacent regions. It provides further evidence that thermoceptive elements are involved in the physiological processes of sleep induction. A significant increase in wakefulness is reported to be induced after local cooling of the hypothalamic structure including POAH in different mammalian species including rats (Hammel et al., 1960; Satinoff, 1964; Fox et al., 1974; Boulant, 1991). Roberts and Robinson (1969) reported that local diathermic warming of preoptic region and anterior
hypothalamus induced relaxation, drowsiness and sleep. The latency of response was found to be 20-150 sec, similar to that obtained by Sterman and Clemente (1962a, 1962b) on electrical stimulation. In most of the above studies POAH warming caused only acute sleep induction, though, Sakaguchi et al., (1979) reported increase in SWS for several hours after hypothalamic warming.

7.1.4 Neuronal studies:

There are studies showing changes in the firing rates of POAH neurons with alteration in the state of S-W and corresponding EEG changes. This region contains two groups of neurons, the discharge rates of which increase either during sleep or wakefulness and associated EEG changes (Mallick et al., 1983; Kaitin, 1984; Szymusiak and McGinty, 1986a, 1986b). The former neurons have been designated sleep-active and the latter wake-active. These neurons are generally assumed to have a cardinal role in the regulation of sleep and wakefulness (Szymusiak and McGinty, 1986a, 1986b).

Experiments carried out on anaesthetized (Lincoln, 1969) and unanaesthetized (Pfaff and Gregory, 1971; Glotzbach and Heller, 1984) rats showed that there are neurons in the POAH and AH area whose firing rates alter with the changes in the state of the EEG between synchronization and desynchronization. About 50% of the neurons showed alterations in their firing rates with changes in the states of consciousness and EEG. Some of the neurons showed an increase in firing rates during synchronization and sleep while others during desynchronization and wakefulness. Findlay and Hayward (1969) reported that 60% of neurons of POAH and AH showed alterations in their firing rates during different states of EEG in rabbits and amongst them a majority showed an increased firing during synchronization.

Mallick et al., (1983) recorded unit activity of preoptic neurons together with cortical EEG in encephale isole cats. They reported that 55% of the neurons showed alterations in their firing rates during transient changes in EEG pattern. Amongst them, 62.5% (majority) showed an increased firing rate during synchronization while remaining showed an increased firing rate during
desynchronization of EEG. The same group also showed that some (and not all) of the POAH neurons related to spontaneous changes in EEG synchronization and desynchronization did show a similar alteration with brainstem stimulation induced changes in the cortical EEG (Mallick et al., 1984; Mohan Kumar et al., 1984).

Kaitin (1984) recorded the unit activity of the POAH neurons in free moving cats and reported 44%, 40% and 16% of the neurons of POAH increased firing rates during REM sleep, slow wave sleep and awake states, respectively i.e., a majority of the neurons showing increased firing rate during sleep period. Szymusiak and McGinty (1986b) reported that a majority of neurons present in the IPOAH region increased in their firing rates during arousal.

7.1.5 Sleep-related c-Fos protein expression in the preoptic-anterior hypothalamic area:

Recently a specific population of neurons in the ventrolateral preoptic nucleus have been identified that show Fos-immunoreactivity after sleep. It was reported that the c-Fos protein, immunoreactive cells were found in a group of ventrolateral preoptic neurons that is specifically activated during sleep (Sherin et al., 1996). It has recently been observed that after sleep, but not after waking, there was increased numbers of c-Fos immunoreactive neurons in both rostral and caudal parts of the median preoptic nucleus (MnPN) and in the ventrolateral preoptic area (VLPO) (Sherin et al., 1996; Gong et al., 2000). The number of Fos-immunoreactive neurons in this region was directly proportional to the number minutes of sleep during the previous hour (Sherin et al., 1996). It was in good agreement with those of electrophysiological studies showing that sleep-active neurons, with firing rates two to three times faster during sleep than during wakefulness, are particularly numerous in the ventrolateral preoptic area. The animals sleeping at ambient temperature (31.5°C), showed significantly more Fos immunoreactive cells in the rostral MnPN compared with animals sleeping at 22°C. In VLPO, Fos IRN counts were no longer increased over waking levels after sleep at the elevated ambient temperature.
7.2 POAH AND THERMOREGULATION:

Normally, the body temperature (Tb) of birds and mammals remains constant except for a daily circadian temperature variation of ±1.0 to 1.5 °C (Fox et al., 1974; Boulant, 1991). POAH constitutes an extremely important thermoregulatory area of mammalian brain. It contains thermosensitive neurons that sense not only core temperature, but also integrate central and peripheral thermal information to elicit the most appropriate response for the given internal and external thermal condition (Reaves and Hayward, 1979; Kobayashi, 1989; Fox et al., 1974; Boulant, 1980,1991). Following types of studies have established the involvement of POAH in thermoregulation.

7.2.1 Lesion studies:

Mechanical or electrolytic lesions of POAH and adjoining areas eliciting chronic hyperthermia is well documented in rats (Gamble and Patton, 1953; Lipton, 1968; Nagel and Satinoff, 1980; Satinoff et al., 1982; Szymusiak et al., 1985), cats (Squires and Jacobson, 1968; Roberts and Robinson, 1969), Kangaroo rats (Sakaguchi et al., 1979; Szymusiak and Satinoff, 1984), opossums (Roberts et al., 1969) and other mammalian species (Boulant, 1991). Even unilateral mPOAH damage in rats (Rudy et al., 1977; Ackerman and Rudy, 1980) is reported to cause acute hyperthermia. Bilateral ablation of preoptic area impairs heat and cold defense abilities in a variety of species including rats (Sherwood et. al., 1954; Satinoff et al., 1976; Van Zoeren and Stricker, 1976) and cats (Squires and Jacobson, 1968). Szymusiak et al. (1991) examined the effects of POAH cell loss caused by N-methyl-D-aspartic acid on thermoregulation in cat. They reported that bilateral POAH cell loss led to an impaired heat defense response with an associated elevation in the hypothalamic temperature threshold for panting from 39.5 °C (pre-lesion) to 41.7 °C (post-lesion). Recently the effect of cell specific damage to the ventrolateral and ventromedial preoptic area on thermoregulatory behavior has been examined. It has been observed that lesions in the ventromedial preoptic nucleus, caused loss of fine tuning of body temperature whereas the loss
of ventrolateral preoptic area neurons do not alter mean body temperature (Lu et al., 2000).

**Differential thermoregulatory responses of medial and lateral POAH**

Szymusiaik and Satinoff (1982) attempted to find out the differential role of medial and lateral POAH in thermoregulation. In unanaesthetized rats, they unilaterally lesioned medial and lateral POAH by anodal current. The hyperthermia accompanied with cold defense responses (such as vasoconstriction of tail, increase in oxygen consumption, shivering and heat conservation postures) was induced after medial POAH electrolytic lesioning. On the other hand, lesion of lateral POAH elicited acute fall in body temperature (Tb), decreased oxygen consumption, inhibition of shivering in cool body temperature and prone body posture. However, unilateral cathodal lesions throughout POAH yielded only hyperthermia. Since both types of lesions *per se* caused tissue damage, the authors reasoned out that the metallic ion deposition due to anodal lesion might have irritated the surrounding neural tissue. Thus tissue at a distance from the electrode tip could mediate the hypothermic action of lateroventral anodal lesion. They further suggested that the lesion induced hyper- and hypothermia was actively generated since the magnitude of both was relatively independent of environmental temperature. Further the differential role of medial and lateral POAH on thermoregulation and the significance of simultaneous alterations in the cortical EEG and S-W have been reported by Alam and Mallick (1990, 1991). They reported that lower dose of marcain (0.2 μl) into medial POAH was effective in inducing hyperthermia whereas it was ineffective when injected into lateral POAH. The hyperthermia was induced when it was injected into medial and lateral POAH both, at higher dose (0.4 μl). The hyperthermia induced due to inactivation of medial POAH by higher dose of marcain was relatively long lasting and magnitude was significantly higher than the lateral POAH.
7.2.2 Stimulation studies:

The strongest evidence for the role of POAH in thermoregulation comes from stimulation studies, the history of which goes back to the classic work of Magoun et al., (1938). They stimulated various locations in the cat forebrain and midbrain with small electrodes heated by high frequency AC current and reported strongest heat loss responses produced by localized warming of the rostral regions of the brain including the POAH. Afterwards, different workers in various species explored POAH as a possible candidate for thermoregulation. In all the studies, localized preoptic cooling increased heat production by shivering and non-shivering thermogenesis (Hellstrom and Hammel, 1967; Jacobson and Squires, 1970; Boulant, 1991). Conversely, preoptic area warming produced cutaneous vasodilation, sweating, panting and other various heat loss behavioral responses (Boulant, 1980, 1991).

7.2.3 Neuronal studies:

Thermosensitive neurons particularly in the mPOAH, which alter their discharge rates to physiological changes in local temperature, have been believed to play a principal role in thermoregulation. Nakayama in 1961, for the first time revealed the presence of thermosensitive neurons in the brain. In almost all the homeothermic as well as poikilothermic vertebrates, thermosensitive neurons have been found in the rostral part of the brain. Thermal stimulation of the hypothalamic area in conscious animals produced adaptive thermoregulatory responses. It is believed that thermosensitive neurons have phylogenetically older origin than the development of autonomic thermoregulatory system, in birds and mammals (Hori, 1991).

Thermosensitive neurons are generally defined as the neurons whose firing rates alter in response to change in local temperature (ranges 35 °C and 41 °C). The two types of thermosensitive neurons have been reported (i) having $Q_{10}$ value more than 2 and/or positive thermal coefficients more than 0.8 imp/sec/°C and (ii) $Q_{10}$ value less than 0.5 and/or negative thermal coefficients more than 6 imp/sec/°C. The former has been termed as warm sensitive neurons while latter as cold
sensitive neurons. The $Q^{10}$ value sometimes exceeded beyond 100, a value outside
the physiological range of metabolic rate. This criterion was derived from
metabolic concepts. There is little evidence that neuronal discharge is related to
metabolic rate in the narrow range of brain temperature regulation. In addition, $Q^{10}$
criterion was liberal for slow discharge rates and was non-linear. On the other
hand, the imp/sec/°C criterion was liberal for fast-discharging neurons, recognizing
about twice as many faster-discharging neurons (10 Hz) compared with other
criteria (Alam et al., 1995). A cell having firing frequency 20 Hz may change only
to 20.8 or 19.4 Hz to meet the imp/sec/°C criterion. Because rate variability is
usually proportional to rate, such small changes may be insignificant. The
limitation of the $Q^{10}$ and imp/sec/°C criteria led to use a third criterion i.e. %
change/sec/°C. This criterion is linear and could be applied to slow and fast firing
neurons. According to this criterion neurons were considered warm sensitive if
imp/sec/°C was greater than or equal to (+) 10% of the basal discharge rate and as
cold sensitive if the % change imp/sec/°C was greater than or equal to (-) 10% of
the basal discharge rate.

7.2.3.1 Neuronal proportions and locations in different species

Thermosensitive neurons have been recorded in several mammalian
species at various locations and in different types of preparations, including
anaesthetized and unanaesthetized animals, in in vitro tissue slices (Boulant and
Demmivelle, 1977; Kelso et al., 1982; Alam et al., 1995) as well as in tissue
cultures (Nakayama et al., 1978). Early single unit studies 1961-1980 indicated
that in the mammalian POAH neuronal population, approximately 30% neurons
were warm sensitive, 10% neurons were cold sensitive and remaining 60% were
temperature insensitive (Fox et al., 1974; Boulant and Dean, 1986; Boulant, 1980,
1991). The location and proportions of thermosensitive (warm and cold) and
insensitive neurons in different species are shown in table: -
## Table 1

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</table>

$Q_{10} > 2$ indicates a significant increase in enzyme activity with a 10-fold increase in temperature. $Q_{10} > 0.5$ indicates a moderate increase. $Q_{10} > -1$ indicates a decrease in enzyme activity with a 10-fold increase in temperature.
Although in the mammalian POAH neuronal population most of the studies showed that approximately 40% neurons are thermosensitive, the percentages of warm and cold sensitive neurons enlisted in the above table showed a different picture. The discrepancies about the percentage of thermosensitive neurons may be attributed to a number of factors such as the species studied, criteria used to establish the thermosensitivity, extent of the anatomical area explored for recording, type and depth of anesthesia at the time of recording etc. The extent of area may affect population of neurons of varying sensitivity because of the presence of heterogeneous population of neurons and their afferent and efferent connections.

7.2.3.2 Thermosensitive neurons in POAH in rats

The number of warm sensitive neurons in the medial POAH is more than that of lateral POAH (Wit and Wang, 1968). Dean and Boulant (1989b) mapped the temperature insensitive neurons in tissue slices of the whole diencephalon. This study indicated that medial POAH contains warm, cold, warm-cold and insensitive neurons whereas lateral POAH contains only warm sensitive and temperature insensitive neurons. It was further reported that POAH contains both inherently and conditionally thermosensitive neurons. They suggested that these neurons might belong to neuronal networks responsible for thermoregulation and other homeostatic systems that are influenced by temperature.

Recently, Curras et al. (1991), for the first time, recorded intracellular neuronal activity in the rat POAH tissue slices and had thrown more light on the thermosensitive neurons. Their study showed that:

1) Intrinsic mechanisms are responsible for neuronal warm sensitivity
2) Cold sensitivity depends on synaptic input from nearby neurons
3) Temperature insensitive neurons employ two opposite, thermally dependent mechanism: a voltage-dependent depolarizing conductance and a higher polarizing sodium-potassium pump.
7.2.3.3 Inherent mechanisms of neuronal thermosensitivity

The Na-K pump is electrogenic and helps in maintaining membrane hyperpolarization. Although Na-K pump is metabolically driven, it can also be affected by temperature. While warming may increase a depolarizing conductance in these neurons, warming also increases the hyperpolarizing Na-K pump, and this serves to stabilize the resting membrane potential and maintain an unaltered firing rate in temperature insensitive neurons. When the Na-K pump is inhibited with ouabain, temperature insensitive neurons increase their firing frequency and become warm-sensitive neurons.

7.2.3.4 Synaptic mechanisms of neuronal thermosensitivity

Preoptic thermosensitive neurons are of two types, warm sensitive and cold sensitive. It has been reported that warm sensitive neurons receive inhibitory inputs from the near by thermo-insensitive neurons. This synaptic input produces inhibitory post-synaptic potentials (IPSP) which can enhance the thermosensitivity of warm sensitive neurons (Cun'as et al., 1991). Griffin et al., 1996 had shown that cooling to 32.6 °C decreased the rate of rise and duration of the depolarizing pre-potential (Fig 1.1), thereby increasing the inter-spike intervals and slowing the firing rate. Since most of the temperature insensitive neurons have slow firing rate
(1-2 impulses/sec), change in the rate of rise and duration of the depolarizing pre-potential has no significant effect on their firing rate. Warm sensitive neurons, however have higher firing rates with shorter inter-spike interval, therefore, the change in the rate of rise and duration of pre-potential significantly affect their firing rates. It can be explained as shown in the figure 1.2 B, that with temperature at 41 °C the average inter-spike interval is 24 ms, but at 32.6 °C, the inter-spike interval is 47 ms. In other words, cooling increases by 23 ms (47-23 ms), the effectiveness of an IPSP to lengthen the inter-spike interval. If a warm sensitive neuron fires at 10 impulses/s, its inter-spike interval is 100 ms. A cold induced increase of 23 ms represents a quarter of this interval and this would slow the firing rate enough to increase the neuron's thermal coefficient. Therefore, the effect of temperature on IPSP effectiveness can contribute to the sensitivity of warm sensitive neurons, Fig 1.2 A illustrates another way in which cooling enhances IPSP effectiveness. At 41 °C, the depolarizing pre-potential is rapid and only allows time for one IPSP to occur during the inter-spike interval. At 32.6 °C, however the slower rate of the pre-potential permits enough time for two IPSPs to occur, causing substantial lengthening of the interspike interval. Thus, during cooling, the slower firing rates allow additional opportunities for IPSPs to slow the firing rate even more.

The cooling-induced increase in IPSP effectiveness is also due to the effect of temperature on the input resistance of warm-sensitive neurons. As with most neurons, cooling causes an increase in the membrane resistance of warm-sensitive neurons. As shown by Ohm's law, the amplitude of the IPSP voltage change is equal to the inhibitory current times the membrane resistance. Therefore, because cooling increases the resistance, it also increases IPSP amplitude and duration thereby slowing the neuronal firing rate.

7.2.3.5 Set-point and temperature regulation

Of several models devised to explain hypothalamic regulation of constant body temperature and its regulation, Hammel (1965) put forward the simplest and most accepted explanation. They suggested that temperature regulation depends on
hypothalamic proportional control with an adjustable "set-point". If preoptic temperature rises above the point (e.g. during exercise), various heat loss responses are initiated to lower body temperature and return the preoptic temperature to its set-point temperature. Conversely, if the preoptic temperature falls below the set-point then various heat generation and retention responses are initiated to raise body temperature and returns the preoptic area set point to maintain the body temperature (Fox et al., 1974; Boulant, 1980, 1991). However, such a concept of single "central set-point" temperature is controversial and many physiologists think of a narrow range of regulated (average body) temperature that represents the composite set point of several thermosensitive areas and different thermoregulatory responses (Boulant, 1991).

Moreover, it is proposed that "set-point" is not a static one, rather it is dynamic and peripheral inputs can alter it. Some preoptic neurons not only sense local temperature but also receive synaptic inputs from afferent pathways of thermoreceptors in the skin, spinal cord and other locations throughout the body (Poletti et al., 1973; Gardner and Phillips, 1977; Boulant, 1980; Hori et al., 1982a, 1982b; Boulant et al., 1989). The neurons of preoptic area integrate central and peripheral thermal signals and control the body temperature by shifting this preoptic set-point (Hammel, 1968; Fox et al., 1974; Boulant, 1991).

7.2.3.6 The role of thermosensitive neurons in thermoregulation

The concept that thermosensitive neurons play a critical role in thermoregulation is based on good correlation between the activity of thermosensitive neurons and ensuing thermoregulatory responses, which can be described as:

(a) Thermally induced changes in thermosensitive neurons and whole body thermoregulatory responses:

Local warming and cooling of POAH, medulla oblongata and spinal cord in mammals evoke heat-defense responses (cutaneous vasodilation, panting and cooling behaviors) and cold-defense responses (shivering and non-shivering...
thermogenesis and heating behaviors), respectively (Hori, 1991). Thermosensitive neurons in the POAH respond not only to local temperature but also to the temperatures in the remote sites (Berner and Heller, 1998). When the effects of different combinations of POAH and the peripheral thermal stimuli were observed on the firing rate responses of POAH thermosensitive neurons in anesthetized animals and on the whole body thermoregulatory effector responses in conscious animals, both responses displayed the multiplicative integration of thermal signals from different parts of body (Hori, 1991). The pattern of integration of thermal signals in both the warm-sensitive neurons and the cold-sensitive neurons was similar to that of heat-defense responses and cold-defense responses, respectively.

(b) Pharmacologically induced changes in the activity of POAH thermosensitive neurons and whole body thermoregulatory responses:

Pharmacological studies have revealed that the direction of changes in the activity of POAH thermosensitive neurons induced by local application of various endogenous and exogenous substances is appropriate with that observed in whole body thermoregulatory responses after injection of these chemicals into the POAH (Hori, 1984a, 1984b; Hori et al., 1987). For instance, microinjection into the POAH of exogenous and endogenous pyrogen (lipopolysaccharide, interleukin-1, interferon-α, tumor necrosis factor), dopamine and Thyroxin Releasing Hormone (TRH) produced hyperthermia (Hori 1991) by inducing a decrease in the heat-defense responses and an increase in the cold-defense responses in the rat (Clark and Lipton, 1983).

Local application of these substances were demonstrated to inhibit the activity of 60-95% (depending on the substances) of POAH warm-sensitive neurons and excite a similar proportion of cold-sensitive neurons (Hori and Nakayama, 1973; Hori et al., 1988a, 1988b; Nakashima et al., 1988; Nakashima et al., 1989; Scott and Boulant, 1984). In contrast, substances such as serotonin, angiotensin II and III, arginine, vasopressin and capsaicin which evoke hypothermia by increased heat-defense and decreased cold-defense of thermoregulation, had the opposite effect on a majority of POAH thermosensitive
neurons (Hori, 1984; Hori and Nakayama, 1973; Hori et al., 1988c; Kiyohara et al., 1984). These hyperthermic and hypothermic substances affected only 20-40% of thermo-insensitive neurons. Poikilothermia-producing peptides like bombesin and neurotensin which suppress the heat-defense and cold-defense responses in warm and cold environments, respectively, reduced the activity of 50-70% of POAH neurons in rats, regardless of the type of their thermosensitivity (Hori et al., 1986).

Table 2. Changes in activity of POAH thermosensitive neurons and whole body thermoregulatory responses to pharmacological stimulation

<table>
<thead>
<tr>
<th>Pharmacological Agents</th>
<th>Heat Defense Responses</th>
<th>Cold Defense Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperthermic Compounds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. LPS pyrogen</td>
<td>Decrease heat defense responses and warm unit</td>
<td>Increase heat defense responses and cold units</td>
</tr>
<tr>
<td>2. IL-1</td>
<td></td>
<td></td>
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<tr>
<td>3. NA</td>
<td></td>
<td></td>
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<tr>
<td>4. DA</td>
<td></td>
<td></td>
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<tr>
<td>5. TRH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothermic Compounds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 5-HT</td>
<td>Increase heat defense responses and warm unit</td>
<td>Decrease heat defense responses and cold units</td>
</tr>
<tr>
<td>2. Angiotensin II &amp; III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Capsaicin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poikilothermic Compounds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Bombesin</td>
<td>Decrease heat defense responses and warm unit</td>
<td>Increase heat defense responses and cold units</td>
</tr>
<tr>
<td>2. Neurotensin</td>
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(c) Responses of POAH Thermosensitive Neurons during thermoregulatory behavior:

Although an attempt to correlate the activity of POAH thermosensitive neurons with on-going autonomic thermoregulation was made in conscious goat (Mercer and Jessen, 1978a, 1978b), dynamic properties of information processing by thermosensitive neurons were not fully elucidated due to the intrinsically "sluggish" properties of the autonomic thermoregulatory responses.
The dynamic response profiles of thermosensitive and non-thermosensitive neurons in the POAH of alert monkeys performing bar press tasks to lower the ambient temperature when exposed to heat i.e. skin cooling behavior (Hori et al., 1987a) have been investigated. If the monkeys performed the high fixed ratio (FR 12-30) bar press tasks in response to a rise in ambient temperature (35-42 °C), the animal was rewarded with a rapid fall in ambient temperature. The population of neurons that changed the firing rate at least in one phase of the skin cooling behavior was significantly higher among thermosensitive neurons than among thermo-insensitive neurons. This indicates that POAH thermosensitive neurons are more closely involved in thermoregulatory cooling behavior than thermo-insensitive neurons. The most frequently observed responses were sustained increase or decrease in activity during the bar press period (the bar press related responses) and the cooling period (cooling phase-related response). The magnitude of bar press-related response was profoundly affected by the animal's motivational state to seek cool air, which was estimated by the behavioral criteria such as the time required to complete the high FR bar press task or the speed of bar pressing.

The cooling phase-related responses was found to be dependent on the rewarding value of the cooling air, indicating that it was not simple sensory response to skin cooling. It was shown that this response became longer when the core temperature of animals was higher and the response lasted for a shorter period when the core temperature was lower. In other words, at higher core temperature the cooling phase related response of POAH neurons continued until the skin cooled down to lower levels. This could be correlated with the hedonic response to thermal stimuli in man: the subjects feel lower skin temperature more pleasantly when core temperature is higher (Cabanac, 1969). The bar press-related response and cooling phase-related response of POAH neurons appear to be somehow related to the drive and reward mechanisms of thermoregulatory cooling behavior, respectively. The dynamic changes in the activity of POAH neurons thus observed indicate that POAH thermosensitive neurons may be involved in phasic information processing which is necessary for the performance of thermoregulatory behavior.
The "warm signal" and "cold signal" network in the CNS and the central control of thermoregulation:

Instead of classical negative feedback models of thermoregulation comprising of single integrator with multiple inputs and outputs, recent conceptual model assumes a multiple thermostat system, in which warm and cold signals activate primarily separate sets of neural networks from thermosensitive neurons to thermoregulatory effectors. There is ample evidence that the controller functions in thermoregulation may be exerted by integrating mechanisms widespread in the POAH and extra-POAH thermosensitive area and presumably these multiple integrators are organized hierarchically (Boulant, 1980; Nakayama, 1985; Simon et al., 1986). Single neuronal studies have revealed that multiple integrators with primarily separate and independent channels from thermosensitive neurons to effectors are collectively grouped into the two sets of a thermoregulatory information processing systems in the CNS, i.e. the warm signal network and the cold signal network.

Thermosensitive neurons in the brain, as described above, receive abundant afferent inputs from other thermoreactive structures in the CNS and skin, while thermo-insensitive neurons rarely respond to thermal stimulation of remote site. This implies that thermosensitive neurons in one site of CNS are synaptically connected to thermosensitive neurons in the other sites, thus forming neural networks of thermosensitive neurons within the brain and spinal cord. Furthermore, a majority of such warm-sensitive and cold-sensitive neurons increase their activity to warming and cooling of remote sites, respectively (Boulant, 1980; Nakayama, 1985; Shibata et al., 1988). For example, thermosensitive neurons respond to temperatures of different sites with the same type (positive or negative) of thermal coefficient. These findings suggest that there are two sets of neural networks for the processing of thermal signals relating to thermoregulation, i.e., one which increases neural activity to a rise in skin and deep body temperatures (warm signal network) and the other which shows the opposite type of response to temperature changes (cold signal network). It is suggested that
the neurons belonging to the warm signal network (i.e., warm-sensitive neurons) activate the heat-defense responses of thermoregulation and the neurons in the cold signal network (i.e., cold-sensitive neurons) facilitate the cold-defense responses of thermoregulation. This view is supported by many examples demonstrating the correlation between the thermally, pharmacologically and neurally mediated changes in the activity of thermosensitive neurons and the ensuing thermoregulatory responses, as described above. The congruency observed in the cold-sensitive RfS neurons in the efferent shivering pathway and shivering responses (Asami et al., 1988; Asami et al., 1988) also support this. It was shown that capsaicin-desensitized animals had impaired responses of autonomic and behavioral heat-defense thermoregulation with intact cold-defense thermoregulation (Hori, 1984). Such dissociation between heat-defense and cold-defense thermoregulation in capsaicin-desensitized animals conforms well with the dissociation observed between warm sensation and cold sensation of the skin and tongue (Hori, 1984). These findings suggest that there is an impaired function of the warm signal network with the intact cold signal network in capsaicin-desensitized animals, thus adding further evidence to support the above hypothesis.

7.2.3.7 Multimodal responsiveness of hypothalamic neurons and thermal and non-thermal homeostatic functions:

The POAH thermosensitive neurons were demonstrated to respond to glucose (Silva and Boulant, 1984) and noxious stimuli (Kanosue et al., 1984). Such overlapping sensitivity to thermal and non-thermal homeostatic parameters has been found as well among hypothalamic neurons that are taken to be primarily involved in food intake and osmoregulation. For instance, about 39% of the neurons in the tissue slices of rat ventromedial hypothalamic nucleus (VMH) possess an inherent thermosensitivity (Hori et al., 1988b, 1988c). It was found that the anesthetized rat those glucose sensitive neurons in the VMH which are considered to suppress food intake showed warm-sensitivity and the glucose-sensitive neurons in the lateral hypothalamus which work to facilitate feeding showed cold-sensitivity (Yamamoto et al., 1981; Nakayama and Imai-Matsumura,
1984). About 70% of neurosecretory cells in the supraoptic nucleus respond to preoptic temperature (Matsumura et al., 1985). The response of neurons subserving the controls of feeding and body fluids seem to explain at least in part, the reduced intake of food during hyperthermia and the facilitation of vasopressin release during heat stimulation, respectively.

The thermoregulatory system itself has no specific effector organs, except for human sweat gland and shares them with other homeostatic systems. Therefore, thermoregulatory actions do not take place in isolation; they are intricately interrelated with the actions of other regulatory systems. Shivering is under the control of the motor system. Non-shivering thermogenesis share the effector organ (brown adipose tissue) with the food intake / energy balance system. The control of dry heat loss by cutaneous vasomotion is affected by changes in the cardiovascular system. Evaporative heat loss is related to osmoregulation. Non-thermal emotional stimuli also affect on-going thermoregulation. Thermal sweating on non-palmer general body skin in men decreases transiently during mental arithmetic and increase in response to pain, noise and other emotional stimuli (Ogawa, 1975). Human sweating can occur during certain conditions of emotional stress. Piloerection in many mammals and grooming in the rat occur in response to emotional as well as thermal stimuli.

7.2.3.8 Spontaneous discharge rate of POAH thermosensitive neurons during natural sleep-wakefulness:

It has been hypothesized that hypothalamic thermosensitive neurons participate in the control of naturally occurring transitions between wakefulness and non-REM sleep. It has been anticipated specially on the basis of thermal stimulation and interaction of sleep-wakefulness with thermoregulatory machinery of POAH studies that a subpopulation of warm-sensitive neurons within the mPOAH should exhibit increased discharge during normal transitions from wakefulness to non-REM sleep, i.e., display a sleep-related discharge pattern while cold-sensitive neurons should display wake related discharge pattern (McGinty and Szymusiak, 1990). Studies of identified POAH thermosensitive neurons during
wakefulness and sleep have been done mainly by three research groups. Parmeggiani and associates have reported that a pooled population of warm-sensitive neurons in cats increased thermosensitivity during SWS compared to wakefulness state (Parmeggiani et al., 1987). Other group showed that warm-sensitive neurons in kangaroo rats display increased thermosensitivity during SWS (Glotzbach and Heller, 1984). McGinty and his colleague have shown that some populations of mPOAH warm-sensitive neurons exhibit increased discharge rate during non-REM sleep compared with waking. This sub-population of warm-sensitive neurons also exhibited significantly increased thermosensitivity when tested during non-REM sleep and majority of cold-sensitive neurons discharged more slowly during non-REM sleep compared with waking. This sub-population of cold-sensitive neurons exhibited decreased thermosensitivity during non-REM sleep (Alam et al., 1995). Therefore, these findings are consistent with the hypothesis that the activation of sleep-related warm-sensitive neurons and the deactivation of wake-related cold-sensitive neurons may play a key role in the onset and regulation of non-REM sleep (McGinty and Szymusiak, 1990).

It has been reported that some of the population of warm sensitive neurons increased whereas the majority of the population of cold sensitive neurons decreased their firing rates during the onset of NREM sleep (Alam et al. 1995). It has been suggested that these warm sensitive neurons play a critical role in the initiation of NREM sleep (Szymusiak 1995) and were termed as hypnogenic warm-sensitive neurons (HWSN) (Nakao et al., 1995). Increased discharge of warm-sensitive neurons and decreased discharge of cold-sensitive neurons was expected to induce heat loss mechanisms (Boulant, 1980). Thus it provides a likely mechanism for the regulated fall in body temperature that normally accompanies sleep-onset and non-REM sleep (Glotzbach and Heller, 1976). It is thought that cold-sensitive neurons receive inhibitory inputs from the near by warm-sensitive neurons (Boulant, 1980; Hori et al., 1980). POAH thermosensitive neurons appear to influence the sleep-waking state, in part, via a mechanism related to the modulation of arousal, located in the brainstem and diencephalon. The rate of spontaneous discharge of wake-related neurons in the midbrain reticular formation (MRF) (De
Armond and Fusco, 1971; Steriade et al., 1982) and posterior lateral hypothalamus (PLH) was suppressed by POAH warming (Krilowicz et al., 1994). Critical forebrain sites for the regulation of sleep and arousal lie in the medial and lateral part of the POAH respectively, within the basal forebrain. Neurons displaying peak discharge rates during waking have also been reported in the magnocellular preoptic area (Szymusiak, 1995). POAH warming has the same effect on the neurons of this area as were observed in cell types of MRF and posterior hypothalamus. It has been reported that brainstem stimulation affected a majority of POAH thermosensitive neurons (i.e. 64 % CSNs and 60 % WSNs). Therefore, it is apparent that some excitatory and inhibitory inputs converge on the thermosensitive neurons of the POAH from MRF in rabbits (Boulant and Demieville, 1977). The pontine tegmental lesioned (posterior to MRF) cats were more susceptible to thermal loads i.e., the thresholds for shivering (heat-gain) and panting (heat-loss) were lowered (Amini-Sereshki and Morrison, 1986). These results suggest that thermoregulation is lowered during REM sleep in such pontine lesioned cats. The role of midbrain reticular formation in inducing EEG desynchronization and wakefulness is well established (Moruzzi, 1972). The afferents to the basal forebrain that are of potential functional importance for sleep-wake regulation arises from throughout the brainstem reticular core (Semba et al., 1988; Jones and Cuello, 1989). It has been suggested that temperature sensitive neurons within mPOAH inhibit arousal-related (putative cholinergic) basal forebrain neurons (Alam et al., 1995). It has also been reported that mPOAH thermosensitive neurons modulate arousal/activating system located outside the basal forebrain. Thermal (De Armond and Fusco, 1971) and electrical stimulation (Szymusiak and McGinty, 1989) of mPOAH has been shown to suppress activity of MRF neurons. The inhibition of wake related neurons in the posterior hypothalamus by the thermal stimulation of mPOAH had also been reported (Krilowicz et al., 1994). These studies showed that there is reciprocal interaction between thermo-sensing neural machinery and wake-related neurons in different brain areas. It was reported that brainstem noradrenergic locus coeruleus neurons participate in sleep-wakefulness (Chu and Bloom, 1973) as well as in thermo-regulating challenges such as panting (Morilak et al., 1987). The posterior hypothalamus, another wake related area, is responsible for the increase in
brain temperature during REM sleep (Denoyer et al., 1991). These evidences showed close interrelation between mPOAH thermosensitive neurons and the neurons of wake related area.

7.2.3.9 Neurotransmitters and Thermoregulation:

(a) Serotonin and Thermoregulation

Overall anatomical, pharmacological and physiological evidences indicate that 5-HT in POAH sub-serves heat production. The microinjection of 5-HT into AH area including POAH is reported to induce a dose-dependent hyperthermia in rats, rabbits, cats, monkeys and birds (Borsook et al., 1977; Myers, 1980). 5-HT receptor-blocking agent is reported to antagonize 5-HT induced hyperthermia (Girault and Jacob, 1979). Cooling of the body is reported to evoke 5-HT turnover within POAH (Myers and Beleslin, 1971; Myers, 1980). Lesion of serotonergic neurons of the POAH by 5, 6-DHT neurotoxin impairs heat conservation at normal Ta as well as heat production in response to a cold challenge in monkeys and rats (Waller et al., 1976; Myers, 1978, 1980). All these observations suggest that 5-HT in the POAH is selectively involved in heat production.

(b) Dopamine and Thermoregulation

Dopamine injection into POAH is reported to induce varying degree of hypothermia in cats (Myers and Yaksh, 1969; Ruwe and Myers, 1978) and rats (Cox and Lee, 1977a, 1977b, 1978). Micrountophoretic application of dopamine on the thermosensitive neurons is reported to decrease the firing rate, mainly of CSNs (Sweatman and Jell, 1977). Heat exposure of cat induced dopamine release in POAH and AH area (Ruwe and Myers, 1978).

(c) Acetylcholine and Thermoregulation

From last three decades, considerable evidences indicate that brain cholinergic systems are implicated in thermoregulation. Injection of ACh and carbachol into POAH has been reported to evoke dose-dependent falls in core temperature at all sites tested within the mPOAH (Hulst, 1972; Poole and
Stephenson, 1979; Imeri et al., 1995; Mallick and Joseph, 1997). The fall in core temperature was associated with increase in tail temperature, locomotor activity and CO₂ elimination (a measure of metabolic rate). Though there are some reports where people have observed hyperthermia evoked by infusion of higher doses of carbachol (3-8 µg) into the medial preoptico-anterior hypothalamic area in different mammalian species (Avery, 1970; Rudy and Wolf, 1972; Myers, 1980).

(d) GABA and Thermoregulation

Central and systemic application of GABA and GABA agonist usually causes a fall in core temperature, while the antagonist induces hyperthermia (Clark and Lipton, 1985; Serrano et al., 1985; Minano et al., 1989; Yakimova and Ovtcharov, 1989). The first indication that the GABA effect might be receptor specific came from the experiments of Serrano et al., 1985, who demonstrated that the GABA-induced hypothermia is not blocked by bicuculline, suggesting that the GABA effect on thermoregulation is not primarily mediated through activation of GABA_A receptors. Since intraperitoneal, as well as intraventricular application of baclofen in doses between 5-10 mg kg⁻¹ (I.P.) and 5-15 ng (I.C.V.) induced hypothermia in mice, it was suggested that GABA_B receptor stimulation is important for the hypothermic effect of GABA (Gray et al., 1987; Jackson and Nutt, 1991). However, GABA_A receptors may also participate in thermoregulation since pretreatment with the GABA_A antagonist bicuculline (3mg kg⁻¹ I.P.) augments the hypothermia induced by low doses of baclofen (1-10 mg kg⁻¹ I.P.). Although it is not clear whether GABA in the mPOAH plays any role in thermoregulation but it has been reported that GABA in mPOAH by acting through its GABA_A receptor modulates sleep-wakefulness (Ali et al., 1999) which seem to be very closely associated with the thermoregulatory machinery present in the mPOAH.

(e) Norepinephrine and Thermoregulation

The history of the part played by the catecholamines (adrenaline and noradrenaline) in the regulation of body temperature dates back to beginning of
this century (Myers, 1980). However, a systemic study regarding the hypothalamic NE in the regulation of Tb was made by Feldberg and Myers (1964). They reported that both adrenaline and noradrenaline not only antagonize the hyperthermic action of 5-HT but also lower the Tb when injected into the cerebral ventricle. In an initial attempt to localize the hypothalamic thermoregulatory structure, they reported that this catecholamine into AH area induced a dose dependent fall of body temperature in cats (Feldberg and Myers, 1965). Subsequently, POAH became a focused area for the investigation of NE mediated regulation of body temperature.

*The Role of Norepinephrine in Thermoregulation*

Microinjection of NE into the POAH was reported to induce a fall in Tb in different mammalian species including cats (Feldberg and Myers, 1964, 1965), monkeys (Myers, 1968; Myers and Sharpe, 1968; Myers and Yaksh, 1969), rats (Lomax et al, 1969; Avery, 1971, 1972; Avery and Penn, 1973; Mallick and Alam, 1992) and other mammalian species (Myers 1980). Mallick and Alam, 1992 had reported that NE in the mPOAH modulates thermoregulatory machinery by acting through α-1 adrenoreceptors. In almost all the above mentioned studies, POAH was one of the sites where injections have been made. The specificity of POAH was further confirmed by experiments where NE was ineffective in inducing hypothermia after its injection into posterior and other areas outside the rostral hypothalamus (Metcalf and Myers, 1978). Quan and Blatteis (1989) reported that NE microdialysed into lateral preoptico-anterior hypothalamic area did not induce any significant changes in core temperature. Cooper et al. (1976a) reported that fall in Tb induced by NE injection into the preoptic area and anterior hypothalamus was independent of ambient temperatures. Acute depletion of NE in the hypothalamus by a 32-64 μg microinjection of guanethidine, caused an increase in Tb in rats (Myers, 1980). It has also been reported that the POAH neurons receive norepinephrinergic inputs from locus coeruleus (Tanaka et al., 1992; Saphier, 1993) and effects of stimulation of locus coeruleus on the POAH neurons could be blocked by adrenoceptor antagonist (Osaka and Matsumura, 1994). It has been
reported that POAH neurons of cold stress rats became much more sensitive to NE (Sun et al., 1997).

On the other hand, there are studies where NE induced a rise instead of fall in Tb, after its injection into POAH/AH area (Cooper et al., 1965; Bligh et al., 1971; Veale and Whishaw, 1976; Rev., Myers, 1980).

Release of Norepinephrine During Thermal Stress

The homeothermic animals exposed to cold maintain a rather constant internal body temperature by a variety of regulatory mechanisms. Exposure to cold increased the circulating concentration of NE as an appropriate biochemical index of the sympathetic activity, which conserves heat by piloerection and vasoconstriction and generates heat by enhancing metabolism. An increase in the concentration of NE in the mPOAH in response to heat exposure has been reported in different mammalian species including rats (Myers and Beleslin, 1971; Metcalf and Myers, 1978; Myers, 1980). On the other hand it has been observed that NE decreases significantly in paraventricular nuclei of the hypothalamus, after 24 hrs of cold exposure, whereas the contents of other monoamines were not altered (Ohtani et al., 1999). The increase of NE concentration in heat exposed animals and decrease of NE concentration in dialysate in cold exposed animals indicate that the NE axons of neurons which terminate in this area are excited due to heat exposure and inhibited due to cold exposure.

The Effect of Norepinephrine on Thermosensitive Neurons

Beckman and Eisenman (1970) reported in cats that NE inhibited and increased the firing rate of WSN and CSN, respectively. Murakami (1973) investigated the responsiveness of preoptic temperature sensitive neurons to iontophoretically applied NA in rats. He found that microintophoretic injection of NE decreased the activity of warm-sensitive neurons and increased the activity of cold sensitive neurons. Further, it has been reported that during cold stress POAH neurons became more sensitive to norepinephrine (Sun et al., 1997). Norepinephrine terminals (Reuss et al., 1999) and adrenoceptors (Palacios et al.,
1987) have been reported to be present on POAH neurons. In in vitro slice preparation study it has been shown that norepinephrine is excitatory to the thermosensitive neurons (Sun et al., 1997). Although it has been reported that α-1 adrenoreceptors are involved in thermoregulation, the sub-types of adrenoreceptors present on these thermosensitive neurons are not known.

7.2.3.10 Temperature Induced c-Fos Expression in Preoptic area Neurons

In the preoptic-anterior hypothalamus, the appearance of the nuclear protein, Fos, was used as a marker of neuronal activation in response to changes in ambient temperature in rats. Neuronal activity in response to acute cold exposure was mapped in the central nervous system of the adult rats using Fos immunostaining (Joyce and Barr, 1992; Baffi and Palkovits, 2000). A single, 3-hour exposure to cold elicited strong Fos-like immunoreactivity in the medial preoptic nucleus. By this technique, pontine and medullary thermosensitive neurons have been first localized and outlined anatomically. The medullary thermosensitive neurons occupy well-demarcated areas immediately ventral and dorsal to the spinal trigeminal nucleus, termed peritrigeminal and paratrigeminal nuclei, respectively. Cold-sensitive neurons were present in the dorsal part of the pontine reticular formation. Topographically, this area corresponds to the 'pontine thermoregulatory area, named on the basis of neurophysiological observations. In addition, thermosensitive neurons were found in the rostral thalamus and zona incerta. The midline thalamic, hypothalamic, dorsomedial, supramamillary and lateral parabrachial nuclei were targets of cold stress-induced noxious stimuli. Fos-positive neurons established specific topographical patterns in the paraventricular, arcuate, central amygdaloid nuclei, and the nucleus of the solitary tract. In hyperthermic, heat-exposed rats, it has been found that the median preoptic nucleus and the medial and lateral preoptic areas had significantly more Fos immunoreactive neurons than control or cold-exposed animals (Scammell et al., 1993; Kiyohara et al., 1995; Baffi and Palkovits, 2000). These observations add to the physiologic evidence that neurons of the preoptic area participate in thermoregulation.
7.3 NEURONAL NETWORKING OF PREOPTICO-ANTERIOR HYPOTHALAMIC AREA

Preoptico-anterior hypothalamic area is connected to a large number of widely distributed forebrain and brainstem structures, most of which in turn also supply inputs to it. Both medial and lateral preoptico-anterior hypothalamus shares most of these inputs and outputs and their pathways. However, they also show difference in some of their neuronal connections (Swanson, 1976; Simerly et al., 1986; Simerly and Swanson, 1988).

7.3.1 Efferent projections:

On the basis of a large number of HRP studies, autoradiographic studies and electrophysiological studies, mPOAH is reported to project to various parts of the telencephalon (Ottersen, 1980; Lind et al., 1982; Chiba and Murata, 1985), diencephalon (Herkenham and Nauta, 1977; Berk and Finkelstein, 1981a, 1981b; Kita and Oomura, 1982; Sawchenko and Swanson, 1983) and brainstem (Aghajanian and Wang, 1977; Swanson et al., 1984; Numan et al., 1985).

Simerly and Swanson (1988) conducted a series of experiments, employing anterogradely transported, lectin *Phaseolus vulgaris* leucoagglutinin (PHA-L) into the medial preoptic nucleus (MPN). The cells within the MPN itself project extensively through the forebrain and brainstem, ascending as far rostral as the infralimbic area of the cerebral cortex and descending to the level of caudal medulla. The output of the MPN appears to be largely ipsilateral and travel through five major pathways: ascending and descending fibers associated with the periventricular zone of the hypothalamus, ascending and descending fibers in the medial forebrain bundle and dorsolaterally directed fibers coursing to or through the stria terminalis and its bed nucleus.

7.3.1.1 Periventricular pathway

Projections from the MPN to more rostral structures near the midline primarily ascend through the preoptic part of the ventricular nucleus and give rise
to clear terminal fields in the anteroventral periventricular nucleus, suprachiasmatic preoptic nucleus, vascular organ of the lamina terminalis, median preoptic nucleus and subfornical organ. Injections of retrograde tracers into each of these structures have been found to label cells in the region of MPN (Palkovits, 1978; Lind et al., 1982; Fulwiler and Saper, 1984; Wiegand, 1984). The MPN also projects massively to the whole extent of the hypothalamic periventricular zone at the level medial and caudal to the MPN (Simerly and Swanson, 1988). These essential descending fibers provide inputs to the preoptic, anterior and posterior parts of the periventricular nucleus as well as to parvicellular parts of the hypothalamic paraventricular nucleus and the arcuate nucleus (Simerly and Swanson, 1988). Inputs to the paraventricular and arcuate nuclei from the medial preoptic area have also been shown with retrograde tract tracing (Koves and Rethelyi, 1976; Zaborszky, 1982; Sawchenko and Swanson, 1983). The ventromedial and dorsomedial nuclei of the hypothalamus appear to receive a majority of their MPN afferents by way of the medial forebrain bundle pathway.

7.3.1.2 Medial forebrain bundle pathway

The largest number of axons from the MPN pass through medial parts of the medial forebrain bundle. Ascending fibers terminate within the anterodorsal, anteroventral and parastrial nuclei of the preoptic region, although the densest rostral terminal field is centered in the ventral part of the septal nucleus. Fibers from the MPN that descend in the medial forebrain bundle give rise to terminal fields in the dorsomedial and ventromedial hypothalamic nuclei as well as to the posterior hypothalamic area and supramammillary nucleus.

The outputs from MPN to the regions caudal to the hypothalamus appear to course primarily through the median forebrain bundle. The ventral tegmental area, periaqueductal gray and peripeduncular nucleus receive strong inputs from the MPN through median forebrain bundle (Conrad and Pfaff, 1976; Swanson, 1976; Beitz, 1982; Chiba and Murata, 1985). Some studies have also reported that a few cells of MPN project to the pedunculopontine nucleus (Swanson et al., 1984; Simerly and Swanson, 1988). The central, dorsal and median nuclei of raphe
receive inputs from the MPN (Aghajanian and Wang, 1977; Simerly and Swanson, 1988). A few PHA-L labeled fibers and terminal buttons were found within the lateral part of the parabrachial nucleus and a few cells within the MPN may in fact project to this nucleus, the strongest preoptic input to the parabrachial nucleus appears to arise within the MPN (Fulwiler and Saper, 1984; Simerly and Swanson, 1988). It is also interesting to note that the MPN appears to project to the nucleus of the solitary tract and to the region of the A1 noradrenergic cell group (Dahlstrom and Fuxe, 1964), both of which may supply noradrenergic inputs to the MPN (Sakumoto et al., 1978; Day et al., 1980). In addition, a small number of fibers and terminal boutons are found in the region of the A5 noradrenergic cell group as well as in the region of the C1 adrenergic cell group.

7.3.1.3 Dorsolateral pathway

The final major projection from the MPN courses through the stria terminalis, with major terminal fields in the encapsulated part of its bed nucleus and in the posterodorsal part of the medial nucleus of the amygdala. Both of these cell groups provide direct massive inputs to the MPN (Chiba and Murata, 1985; Simerly et al., 1985; Simerly and Swanson, 1986). The MPN also appears to provide inputs to cells along the lateral border of the bed nucleus and to adjacent parts of the substantia innominata.

7.3.1.4 Differential projections of each subdivision of the MPN

Each part of the MPN does not contain a homogenous population of cells and there appears to be some overlap in the distribution of cell types for each of the three subdivisions (Simerly et al., 1986). Nevertheless, both the inputs to the nucleus and the distribution of neurotransmitter-specific cells are largely localized to a single subdivision, suggesting that each part of the MPN receives a unique profile of afferent information, which may be relayed along equally specific projection pathways.
Characteristic projections of the medial preoptic nucleus medial (MPNm):

Cells within the MPNm project to nuclei within the periventricular zone of the hypothalamus, including the anteroventral periventricular nucleus, the paraventricular, arcuate nuclei and each part of the periventricular nucleus. Projections from the MPNm to the brainstem appear to be relatively sparse, although many terminal boutons are found within the medial part of the supramammillary nucleus and more caudally, within the laterodorsal tegmental nucleus (Simerly et al., 1986).

Characteristic projections of the medial preoptic nucleus central (MPNc):

The MPNc appears to project more strongly through the dorsolateral pathway to the encapsulated part of the bed nucleus of the stria terminalis and the posterodorsal part of the medial nucleus of the amygdala. A strong projection to the ventro-lateral septal nucleus is also evident. Within the hypothalamus, the MPNc provides inputs to the anteroventral periventricular nucleus and to the arcuate nucleus, although they are less dense than those from the MPNm. MPNc sends many fibers to the ventral premammillary nucleus, a projection that is sparse or lacking from the other two subdivisions of the MPN. PHA-L injections centered in the MPNc also label many fibers and terminal boutons in the ventral tegmental area of the midbrain as well as in the laterodorsal tegmental nucleus of the pons (Simerly et al., 1986).

Characteristic projections of the medial preoptic nucleus lateral (MPNl):

The major ascending projection of the MPNl appears to be a massive input to the ventral part of the lateral septal nucleus. It has been suggested that the majority of MPN efferents to the lateral septal nucleus arise from cells within or near the MPNl (Ishikawa et al., 1986). Most efferent fibers from the MPNl descend through the median forebrain bundle and give rise to terminal fields that are particularly dense within the dorsomedial nucleus of the hypothalamus, the periformal region of the lateral hypothalamic area, the posterior hypothalamic
area and the peripeduncular nucleus. The most widespread projections from the MPN to the brainstem appear to arise primarily from cells within the MPN.

7.3.1.5 Projections of adjacent preoptic nuclei

The medial preoptic and anterior hypothalamic areas were often thought of, more-or-less, a homogenous continuum that lack distinct cell groups or nuclei. But later it was observed that these regions are indeed highly differentiated (Simerly and Swanson, 1988). There are several features shared by the projections from the anterodorsal, anteroventral, parastral and anteroventral periventricular nuclei of the preoptic region. Each of these cell groups appears to project to the parvicellular division of the paraventricular nucleus of the hypothalamus, the dorsomedial hypothalamic nucleus, the posterior hypothalamic area and the supramammillary nucleus. On the other hand, a few regions receive inputs from two or three of these nuclei, but not from the other(s). For example, a subfornical organ and the supraoptic nucleus receive afferents from the anteroventral periventricular and parastral nuclei but not from the anterodorsal or anteroventral preoptic nuclei. The subfornical organ and the median preoptic nucleus also appear to receive substantial inputs from the anteroventral periventricular nucleus (AVPv) (Simerly and Swanson, 1988).

7.3.2 Afferent projections:

The medial preoptic nucleus receives inputs from widely distributed areas throughout the forebrain and brainstem. These inputs appear to be topographically distributed within the three-cytoarchitectonic subdivisions of MPN. With a few exceptions, each major region of the hypothalamus provides inputs to the MPN; projections from nuclei within the periventricular zone end primarily in the medial part of the MPN, while inputs from the lateral zone are mainly confined to the lateral part of the nucleus. Although most cell groups in the medial zone of the hypothalamus end preferentially in the lateral part of the MPN, projections from the anterior and ventromedial hypothalamic nuclei are apparently more widespread within the nucleus. However, the central part of the MPN does not appear to
receive a direct input from the ventromedial nucleus. The MPN also receives strong inputs from the telencephalic regions including the amygdala, ventral subiculum and the ventral part of the lateral septal nucleus, all of which end preferentially in the lateral part of MPN. In contrast, fibers arising in the encapsulated part of the bed nucleus of the stria terminalis appear to end in the central part of the MPN and in the adjacent regions of the medial subdivision. However, the nucleus appears to receive relatively sparse inputs from infralimbic and insular cortical areas, the nucleus accumbens and the substantia innominata. Finally, ascending serotonergic and nonserotonergic projections from the raphe nuclei appear to terminate primarily in the lateral part of the MPN, whereas inputs from regions containing adrenergic and noradrenergic cells (nucleus of solitary tract, locus coeruleus and A1 regions) are chiefly distributed to the central and lateral part of the nucleus. Other brainstem regions that appear to provide modest inputs include the ventral tegmental area, the central tegmental field, the periaqueductal gray, the pedunculopontine nucleus and the peripeduncular nucleus.

7.3.2.1 The cells of origin of afferent projections to the MPN

In general retrogradely labeled cells were found throughout the hypothalamus and were usually not organized into distinct recognizable clusters. Within the periventricular zone of the hypothalamus both the arcuate and anteroventral periventricular nuclei contained relatively high densities of retrogradely labeled cells. Within the medial zone of the hypothalamus rather dense cluster of retrogradely labeled cells were found in the ventrolateral part of the ventromedial nucleus and caudal to this in the ventral premammillary nucleus. Saper et al., 1976, reported that most of the projections from the ventromedial nucleus to the medial preoptic area arise from its ventrolateral part. The clear input to the MPN from the septal region appears to arise in the ventral part of the lateral septal nucleus, especially from the small, densely packed cells that protrude ventromedially near the anterior commissure. Most retrogradely labeled cells in the amygdala were confined to the posterodorsal part of the medial nucleus and to the amygdalohippocampal area (Berk and Finkelstein, 1981b; Kita and Oomura, 1982;
Simerly and Swanson, 1986). Electrophysiological studies also suggest that amygdala projects to the medial peroptic area (Boulant and Demieville, 1977; Renaud, 1979). In addition to the direct afferents from the medial amygdala and amygdalohippocampal area, the MPN also receives influence from bed nucleus of stria terminalis by way of a relay through the bed nucleus (Krettek and Price, 1978; Simerly and Swanson, 1986). There are reports suggesting that there are projections from the ventral tegmental area (Simon et al., 1979; Beckstead et al., 1979) and pedunculopontine nucleus (Simerly and Swanson, 1986) to the medial preoptic area. In view of the connections with extrapyramidal motor nuclei (Saper and Loewy, 1985) and pedunculopontine nucleus (Grillner and Wallen, 1985) which were thought to be involved in the modulation of locomotor activity associated with the behavioral responses, those functions might be influenced by the MPN. The distinct projection has also been observed from just dorsal and lateral to the medial lemniscus in the junctional region between the midbrain and diencephalon. These cells appear to lie within a cytoarchitectonically recognizable differentiation of the central tegmental field (Berman, 1968) and are continuous laterally with the peripeduncular nucleus. Although, it is not clear whether this retrogradely labeling bands lies within a homogenous nuclear region, or whether it lies within the peripeduncular nucleus and a more medial differentiation of the reticular formation. More so the electrophysiological studies have shown that medial preoptic area receives direct or indirect projections from the reticular core in rabbit (Boulant and Demieville, 1977). In addition to its projection to the MPN, the peripeduncular nucleus appears to share bi-directional connections with the ventromedial nucleus of hypothalamus (Saper et al., 1976; Kita and Oomura, 1982). It has also been suggested that MPN receives inputs also from raphe nuclei (Moore et al., 1978; Loewy, 1981, Simerly et al., 1984a, 1984b). In brief, it can be said that serotonergic inputs appear to arise exclusively from the dorsal and median nuclei of the raphe (B7 & B8 cell groups of Dahlstrom and Fuxe, 1964) and the B9 cell group adjacent to the medial lemniscus, while non-serotonergic inputs arise from the central linear nucleus of raphe and nuclei raphe magnus and obscurus (Simerly and Swanson, 1986). It has also been observed that MPN receives afferents from the locus
coeruleus, nucleus of solitary tract and the region of the A1 noradrenergic cell group (Jones and Moore, 1977; Ricardo and Koh, 1978; Norgren, 1978). However, only inputs from the nucleus of the solitary tract and A1 region appear to be noradrenergic (Sakumoto et al., 1978; Day et al., 1980).

7.3.2.2 The distribution of afferent projections within the MPN

The distribution of inputs to the MPN can be divided into two main categories: first, those projecting primarily to the cell dense medial and central parts of the nucleus and second, those projecting primarily to the cell-sparse lateral part. The cell-dense regions of the MPN receive inputs from nuclei of the periventricular zone of the hypothalamus, namely the anteroventral periventricular, paraventricular and arcuate nuclei and all parts of the periventricular nucleus as well. The medial part of the MPN also receives substantial inputs from the anterior and ventromedial nuclei of the hypothalamus. The projection from the anterior hypothalamic area distributes uniformly throughout the nucleus. The afferents from brainstem regions containing noradrenergic cell groups appear to end primarily within the medially located, cell dense parts of the MPN (Swanson and Hartman, 1975; Simerly et al., 1986). The only input to the MPN that appears to end preferentially in the central part of the nucleus arises from cells in the encapsulated part of the bed nucleus of the stria terminalis.

The primary inputs to the cell-sparse lateral part of the MPN appear to arise in nuclei of the medial zone of the hypothalamus and in limbic regions of the telencephalon. The lateral part of the MPN receives a major input from the ventral part of the lateral septal nucleus and a somewhat less dense input from the ventral subiculum. In view of the strong projections from the ventral subiculum to the ventral part of the lateral septal nucleus (Swanson and Cowan, 1977), the relative strength of these inputs to the lateral part of the MPN suggest that the major route by which information from the hippocampal formation reaches the MPN is by way of the ventral lateral septal nucleus. The lateral part of the MPN also receives a substantial input from the amygdalohippocampal area. It has also been reported that MPN also receives inputs from the nuclei of raphe. Immunohistochemical
findings showed that serotonergic afferents from the dorsal nucleus, median nucleus and B9 cell group end primarily within the lateral part of the MPN (Simerly et al., 1984a, 1984b).

### 7.3.3 Neurotransmitter Specificity of Cells and Fibers in the Medial Preoptic Nucleus:

The MPN appears to be innervated by several of the neuro-chemically defined systems and most of these show a characteristic differential distribution within the nucleus that correlates with the three cytoarchitectonically defined subdivisions.

(a) **Dopaminergic Cells and Fibers**

The only monoaminergic cell bodies identified in the medial preoptic area cross-react with antisera to tyrosine hydroxylase (TH), indicating that they are dopaminergic cells. Neither DBH nor 5HT-immunoreactive cell bodies were ever found in any part of medial area, although few TH-stained cells were seen ventrolateral to the MPN. The TH-stained cells were located mostly in the periventricular zone and several cells were found in the region between the MPN and the optic chiasma. A few immunoreactive cell bodies were also found in the dorsal part of the medial preoptic area. A moderately dense halo of the immunoreactive fibers was seen around the MPN as a whole. A small numbers of stained fibers were found within the nucleus itself, although the MPN medial contained a slight higher density than did either of the other two subdivisions.

(b) **Serotonergic Cells and Fibers**

The greatest density of 5-HT stained fibers were found in the MPN lateral and appeared to reach the nucleus primarily via medial forebrain bundle. In contrast low density of 5-HT-immunoreactive fibers was found in the MPN medial and almost none was found in the MPN central. The 5HT-immunoreactive cell bodies were never found in any part of the mPOAH.
(c) Noradrenergic Cells and Fibers

The immunohistochemical studies of the catecholamine synthesizing enzymes, TH and DBH in the mPOAH have shown that TH is present in the perikarya and processes in the anteroventral periventricular region of the mPOAH, while DBH was only seen in the fibers and terminals. TH is the key enzyme for the synthesis of the three catecholamines: dopamine, norepinephrine and epinephrine and DBH is necessary to introduce β-hydroxy group of norepinephrine and epinephrine. TH immunoreactivity, therefore is an indicator of any catecholaminergic neuron and DBH for noradrenergic and adrenergic neurons. A halo of fine, varicose DBH immunoreactive fibers surround the MPN as a whole. Such fibers are moderately dense in the MPN medial and MPN central and quite sparse in the MPN lateral. Such observations showed that no noradrenergic cell was found in the mPOAH, however, only noradrenergic fibers were detected.

(i) Classification of noradrenergic cell groups:

A1-A7 cell groups, based on their efferent projections have been classified into two major sub-groups:

(I) Lateral Tegmental (LT) Group (A1-A3, A5 & A7)
(II) Locus Coeruleus (LC) Group (A4 & A6)

The LT system primarily projects to the spinal cord, brainstem and basal forebrain whereas LC system projects mainly to spinal cord, cerebellum, thalamus and cerebral cortex (Moore and Bloom, 1979; Holets et al., 1988).

Lateral Tegmental Group:

It consists of two NA cell groups:

(a) Medullary Group: It is further divided into two cell groups.

* A1 Cell Group: It is the first and largest accumulation of NA neurons and are found scattered ventral and lateral to the lateral reticular nucleus. The cells are large, multipolar with few long dendrites and predominantly oriented in a plane transverse to the long axis of brainstem.
- **A2 Cell Group**: It lies in the dorsal and medial part of the medulla oblongata, in the nucleus of the solitary tract and dorsal motor nucleus of the vagus with a few in the tegmentum. Typically the cells are smaller than A1 cells and with less dendritic arborization (Moore and Card, 1984).

- **A3 Cell Group**: This cell group lies in the dorsal accessory olivary nucleus.

(b) **Pontine Group**: The pontine group consists of A5 & A7 cell groups.

- **A5 Cell Group**: It includes neurons located ventral to locus coeruleus and within the lateral reticular nucleus.

- **A7 Cell Group**: It is present in the rostral pons, medially to and within the ventral nucleus of lateral lemniscus.

(ii) **Locus Coeruleus Group**:

This group consists of cells in the LC proper as well as the A4 cell group and subcoeruleus cell group.

- **A4 Cell Group**: It is dorsolateral extension of the A6 cell group lying in the lateral part of the roof of the 4th ventricle.

- **A6 Cell Group**: It comprises of cells densely packed within the LC proper and intermingled with cells within subcoeruleus.

(ii) **Noradrenergic projections to the mPOAH**:

The noradrenergic projections innervating to the hypothalamic nuclei specially to the mPOAH are mainly from LT and LC neuronal groups (Fuxe, 1965; Moore and Bloom, 1979). In preoptic region, medial preoptic area contains dense whereas lateral preoptic area contains a light to dense innervation of NE axons (Moore and Bloom, 1979). A6 adrenergic neurons in the LC (Jones and Moore, 1977) and A1 noradrenergic neurons in the ventrolateral medulla (VLM) (Osaka and Matsumura, 1994), project to the entire forebrain, including the mPOAH. It has been reported that mPOAH receives excitatory adrenergic inputs mainly from locus coeruleus and ventrolateral medulla of the brainstem (Tanaka et al., 1992, Saphier, 1993). Locus coeruleus is a component of the reticular activating system.
(Aston-Jones and Bloom, 1981) and electrical stimulation studies had shown that this area influenced the POAH neurons. Osaka and Matsumura (1994) have reported that POAH neurons are responsive to the electrical stimulation of these two areas. They have observed that almost equal proportion of sleep and wake-related neurons are inhibited and activated respectively by electrical stimulation of locus coeruleus.

(iii) Adrenoreceptors and their subtypes:

The stimulation of noradrenergic neurons in both the peripheral and central nervous system produces a wide variety of physiological effects by modulation of adrenergic neurons. These changes are brought about by the activation of different kind of adrenoceptors. A number of different criteria have been used for classification.

Pharmacological Criteria:

Ahlquist, in 1948, suggested that the chemical mediator released from sympathetic nerve terminals acted on distinct types of adrenoreceptors, which he called α and β, that had different affinities for adrenaline and noradrenaline and that could be characterized pharmacologically by the use of specific agonist and antagonists.

Autoradiography:

Localization of adrenoreceptor subtypes by light microscopic autoradiography was reported by Palacios and Kuhar (1980) and Young and Kuhar (1980a, 1980b). This technique provides another criterion, that of pattern of distribution for the identification of receptor subtypes. Autoradiography does not have sufficient resolution to distinguish between presynaptic and postsynaptic receptors. Recently, anterograde and retrograde axonal transport of receptors have been demonstrated by measuring accumulations of specific binding sites above and below a ligature (Laduron, 1980). Such axonally transported receptors have been taken as evidence for presynaptic receptors.
(iv) Molecular characterization of adrenoreceptors:

The use of specific affinity labels and of autoantibodies led to isolation of catecholamine receptor subtypes and determination of their molecular subunits. The radiation inactivation of target size analysis have shown that some adrenergic receptor subtypes are monomers and others are dimers (Venter et al., 1984).

Application of various techniques have established the existence of different types of adrenoreceptors that are summarized as follows:

<table>
<thead>
<tr>
<th>α-1 Adrenoreceptor</th>
<th>α-2 Adrenoreceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Location of receptor on neurons</strong></td>
<td><strong>Post-synaptic</strong></td>
</tr>
<tr>
<td>Name given on the basis of pharmacological study</td>
<td>α_{1A}</td>
</tr>
<tr>
<td>Name given on the basis of molecular study</td>
<td>α_{1A}</td>
</tr>
<tr>
<td>Signal Transduction mechanisms</td>
<td>IP_{3}/DAG</td>
</tr>
</tbody>
</table>

(v) Noradrenergic receptors in the mPOAH:

α-1 adrenoreceptor in preoptic area: The density of α-1 adrenoreceptors have been reported to be very low in the mPOAH despite the fact that mPOAH receives noticeable norepinephrinergic innervations from LC and VLM (Palacios et al., 1987). Besides this, it has also been reported that the density of α-1 receptors exhibited a diurnal changes (Weiland and Wise, 1990).

α-2 adrenoreceptor in preoptic area: A high concentration of α-2 adrenoreceptors have been reported in some periventricular areas including medial preoptic area and some part of the lateral preoptic area (Young and Kuhar, 1980a, 1980b).

β-adrenoreceptor in preoptic area: A low distribution of β-adrenoreceptors have been reported in the mPOAH. A relatively higher concentration was present in the periventricular nucleus (Palacios and Kuhar, 1980). Like α-1 adrenoreceptors, density of β-adrenoreceptors also exhibited diurnal rhythm (Weiland and Wise, 1990).
(vi) Differential role of adrenoreceptors in the modulation of mPOAH neurons:

The activation of mPOAH neurons in response to the electrical stimulation of an adrenergic area, ventrolateral medulla in rats has been observed (Tanaka et al., 1992). This response of mPOAH neurons was blocked after microiontophoretic application of phentolamine (α receptor blocker) but not by timolol, (β receptor blocker), implying mediation through α adrenoreceptors. Bai and Renaud, in 1998, reported that α-1 adrenoreceptors have depolarizing whereas α-2 adrenoreceptors have hyperpolarizing effect on mPOAH neurons. Similarly it has been reported that β adrenoreceptors have hyperpolarizing effect on the mPOAH neurons (Osaka and Matsumura, 1995).

(c) GABAergic Cells and Fibers:

In the rostral part of mPOAH, prominent aggregations of cells labeled with cDNA probe for GAD mRNA were observed in the septo-hypothalamic and parastrial nuclei. After cholchicine pretreatment, GAD-IR cells were also detected in these areas (Okamura et al., 1990). Dorsal to mPOAH, considerable numbers of cDNA labeled cells were observed in the bed nucleus of stria terminalis. Prominent aggregations of cDNA-labeled cells were also observed in the MPN. These labeled medium size cells seemed to represent a major part of the total cell population of the nucleus. Immunocytochemistry revealed dense plexuses of GAD-IR fibers and terminals, as well as GAD-IR cell bodies in the MPN. Further caudally, the cDNA labelled cells of the MPN merged dorsolaterally with labeled cell aggregations in the striohypothalamic nucleus and the bed nucleus of the stria terminalis. A lower density of cDNA-labelled cells was visualized in the preoptic periventricular nucleus (Okamura et al., 1990; Gritti et al., 1994). It has recently been reported that sleep-active GABAergic neurons in the ventrolateral preoptic area, projects to histaminergic tuberomammillary neurons (Sherin et al., 1996; Sherin et al., 1998).
(d) Cholinergic Cells and Fibers:

The neurons that are immunohistochemically positive to acetylcholine (ACh) synthesizing enzyme choline-acetyltransferase (ChAT) occur as a continuous series of cell clusters that are contained within several structures within basal forebrain. From rostral to caudal, they are found in the medial septum, the vertical and horizontal limb of diagonal band of Broca, magnocellular preoptic area, subpallidal substantia innominata and the peripallidal nucleus basalis of Meynert. ChAT-positive neurons are large and multipolar. Several fiber systems traverse these regions including the medial forebrain bundle, anterior commissure etc (Gritti et al., 1994).

Anatomically, the mPOAH and magnocellular preoptic areas are different. As with other periventricular portions of the hypothalamus, the cells in this area are medium to small in size and are densely packed. Extensive lists of neuropeptides, hormone releasing factors and neuromodulators have been localized to mPOAH neurons but no cholinergic neurons are found within these nuclei (Gritti et al., 1994; Szymusiak, 1995).

(e) Neuropeptide Y:

A considerable amount of Neuropeptide Y (NPY)-stained fibers were found in the MPN. They are moderately dense in both the MPN central and MPN medial and somewhat more dense in the lateral part of the MPN medial. NPY-immunoreactive fibers were found in both the periventricular zone and median forebrain bundle near the MPN.

(f) Cholecystokinin:

The highest density of Cholecystokinin (CCK)-stained fibers have been reported in the mPOAH as a whole. The posterodorsal preoptic nucleus contains a high density of CCK-stained fibers. The moderately dense plexus of CCK-
immunoreactive fibers has also been found in the MPN medial but MPN lateral contains few CCK-stained fibers.

\( g \) \textit{Substance P:}

Each subdivision of the MPN contained a dense distribution of Substance P (SP)-immunoreactive fibers. The MPN medial contained a dense plexus of such fibers and appears to represent the strongest input to the MPN medial. In contrast, fewer SP-immunoreactive fibers were found in the MPN lateral and the MPN central was nearly free of them.

8. \textit{INVOLVEMENT OF NEUROTRANSMITTER IN THE REGULATION OF SLEEP-WAKEFULNESS AND BODY TEMPERATURE EITHER INDEPENDENTLY OR SIMULTANEOUSLY}

The role of medial preoptico-anterior hypothalamic area in sleep-wakefulness and body temperature has been well documented earlier in this chapter. However, it is not unequivocal if the changes in those functions have a causal relationship. Some reports indicated that both might be dependent (McGinty and Szymusiak, 1990) while others emphasize that changes in one are likely to be independent of simultaneous alterations in other. Therefore, it is evident that any factor, which changes one of the functions other will be affected as well. It has been illustrated earlier in this review that norepinephrine, acetylcholine, GABA, histamine and serotonin all these neurotransmitters in the mPOAH strongly modulate both sleep-wakefulness or body temperature either independently or concurrently. In spite of the fact that mPOAH modulates both the functions simultaneously, to establish the rationale behind the relationship between those changes, it was necessary to study the effects of different neurotransmitters simultaneously on those changes. Based on microinjection of agonist of one of the receptors in the presence of antagonist of another, it has been proposed that while adrenergic inputs in the mPOAH might affect sleep, wakefulness or body temperature independently (Mallick and Alam, 1992), the cholinergic inputs modulate these
functions simultaneously (Mallick and Joseph, 1997). Subsequently, it has also been reported that aminergic and cholinergic inputs in the mPOAH interact for optimum regulation of sleep-wakefulness and body temperature (Mallick and Joseph, 1998). GABA, an inhibitory neurotransmitter, is reported to be present in almost half of all synaptic boutons in the hypothalamus (Decavel and Van den Pol, 1990). As has been mentioned earlier that separate groups through their independent studies had showed that GABA-ergic input in the mPOAH affected sleep-wakefulness and body temperature but GABA mediated effects on their temporal relationship (sleep-wakefulness vs brain and body temperature) is still not known and it needs further elaborate study.

It has been reported that microinjection of NE-ergic agonist and antagonist into the mPOAH induces wakefulness as well as hypothermia. From the results of single injection of either agonist or antagonist, it can probably be said that all the adrenoreceptors (α-1, α-2 and β) may be involved in the regulation of S-W and body temperature, however, it will be a premature and over simplified conclusion. Therefore, to find out the precise role of these adrenoreceptors in the regulation of S-W and body temperature, Mallick and Alam (1992) did a remarkable heroic work and they designed an experiment of cocktail method for the first time. They studied the effects of different adrenergic receptor agonists and antagonists individually as well as in combination into the medial preoptic-anterior hypothalamic area. The results suggested that norepinephrine induced, preoptic area mediated influence on the body temperature primarily through α-1 receptors while the sleep and wakefulness were regulated by α-2 and β adrenoreceptors, respectively. Furthermore, based on this proposition Osaka and Matsumura (1995) had shown that sleep related neurons in the mPOAH were inhibited by α-2 adrenoreceptos. It was not known whether thermosensitive neurons in the mPOAH possess α-1 adrenoreceptors. These studies demonstrated the dissociated effects of NE on S-W and body temperature. The effect of acetylcholine in the mPOAH as reported by Mallick and Joseph (1997) is unlikely to be a dissociated effect.
The role of GABA-ergic inputs in the mPOAH simultaneously on sleep-wakefulness and body temperature was not known. Since both these phenomena follow circadian rhythm and they may influence each other, the effects on both these functions needed to be studied simultaneously. It has been reported that local microinjection of GABA and its agonist and antagonist in this area modulate body temperature. However, since it has been mentioned above in this review that there are warm and cold sensitive neurons in the mPOAH, which are affected by local and peripheral temperatures. In order to understand the mechanism of action of GABA in temperature regulation at the cellular level it was necessary to study the role of GABA on individual thermosensitive neurons in in vivo preparations.
It is known that GABA by acting through GABA-A receptors in the mPOAH induced sleep and hypothermia. However, the role of GABA-A receptors in the simultaneous regulation of sleep-wakefulness, brain and body temperature was not known.

In the mPOAH, GABA through GABA-A receptors induced hypothermia. The role of GABA-A receptor in vivo at the cellular level on the thermosensitive neurons was not known.

Since norepinephrine in the mPOAH induced hypothermia by acting through its α-1 adrenoreceptors, it is expected that thermosensitive neurons in mPOAH possess α-1 adrenoreceptors, however it needed to be confirmed.

Type of inputs to preidentified thermosensitive neurons in the mPOAH from the brainstem, a wake inducing area was not known.
OBJECTIVE

1. To study the role of GABA-ergic inputs to the mPOAH for simultaneous regulation of sleep-wakefulness and body temperature.

2. To study the subtype of adrenergic and GABA-ergic receptors on the thermosensitive neurons in the medial preoptico-anterior hypothalamic area.

3. To study the input from the midbrain reticular formation (MRF), a wake and EEG desynchronization area, on the thermosensitive neurons in mPOAH.
MATERIAL & METHODS
The improvement in electrophysiological methods together with stereotaxic instrumentation and increased anatomical knowledge, have made possible the recording of summated potential, single neuronal activity, precise local electrical stimulation and microinjection of agonist and antagonist of neurotransmitters, into many local areas of the brain in anaesthetized as well as freely moving normally behaving animals. The idea of exploring the brain of conscious animals with implanted electrodes is not new. Sellier and Verger (1898) were apparently the first to make bipolar electrolytic lesions using two insulated needles bound together. It was Ewald, in 1898, who first used the method of electrical stimulation in freely moving animals. There are two types of techniques that may be used for brain stimulation:

1. Remote control, in which a receiver activated by induction or by radio is implanted beneath the scalp, with a terminal lead ending within the brain tissue. This technique has an advantage in that there are no leads piercing the skin of the animal. But it has limitations: in general, only one cerebral point can be stimulated; monitoring of stimulation is not possible; intensity of stimulation depends on the receiving signal, which may vary with changes in orientation of the receiving antenna.

2. Direct control, in which electrodes are implanted within the brain, with terminals ending outside the skull for electrical connection. Some of the techniques are very simple. A phonograph needle coated with baked enamel and pushed through the skull can be used for stimulation and for recording the activity of the brain (Hoagland, 1940). Sleeve-shaped guides may be fixed into the skull, and later electrodes or cannulae may be introduced through the sleeves (Lilly, 1958).
Several ingenious devices were in existence which allowed drug application to the areas of tissue only a fraction of a square millimeter in size, such as microinjection, microdialysis, pressure injection, Kuffler's hooked wire loop technique etc. Even existence of these methods, however, does not allow the refinement necessary to identify the receptors present on the specific neurons. A technique appropriate to study the synaptic pharmacology was first realized by Nastuk, 1953. Later it was developed by del Castillo and Katz, 1955. The technique consisted essentially of the iontophoresis method, i.e. movement of charged particles produced by an electric current, restricted to a micropipette with a tip diameter of 1-4 μm. Independently on the same line, Suh et al., 1936 had ejected acetylcholine from micropipette by the use of an applied voltage into different regions of the hindbrain. This work was not known to researchers until relatively recently.

Curtis and his colleagues soon adopted the new technique, now appropriately named microiontophoresis or microelectrophoresis for injection of drugs in the vicinity of neurons as well as recording of neuronal activity of mammalian nervous system. The experiments of Curtis and coworkers, however, involved an important modification of the original method and for the first time this group used multibarrel micropipette.

The recording of electrical potential from the intact skull was first demonstrated by a German psychiatrist, Hens Berger (1929). In this study, the electrical activities from the brain and muscles were recorded. The brain electrical activity was recorded with plate electrodes (EEG) as well as at single neuronal level. The neuronal activities were correlated with changes in body temperature recorded from the brain as well as rectum. The presence of receptors on the neurons was established microiontophoresitically. The projection to the neurons was investigated by unilateral stimulation of a specific region in the brain. The technique will be discussed in detail in the following pages.
Recording in Anaesthetized Rats:

In anaesthetized rats, in order to monitor the neuronal activities in response to drugs and brainstem stimulation following parameters were recorded:

a. The electroencephalogram (EEG)
b. Single neuronal spike
c. The brain (T_br) and rectal temperature (T_rec)

Recording in Freely Moving Normally Behaving Rats:

In free moving rats, to see the effects of drug microinjection on S-W and body temperature, the following parameters were recorded:

a. The electroencephalogram (EEG)
b. The electro-oculogram (EOG)
c. The electromyogram (EMG)
d. The brain (T_br) and rectal temperature (T_rec)

I. MATERIALS USED TO COLLECT THE DATA:

Experimental Animals:

The experiments were performed on male Wistar rats, 250-300 gm. Rats have been extensively used in neuroscience research, especially for sleep-wakefulness and thermoregulation studies. The use of rats in this study offered the following advantages:

a) easy availability
b) easy to handle
c) they have polycyclic sleep patterns and hence are suitable for this type of study
d) sufficient size of the brain and skull to implant several electrodes
e) medial preoptico-anterior hypothalamic area is reasonably demarcated
f) stereotaxic apparatus and atlas are available
g) sufficient literature on physiological and behavioural (sleep-wakefulness and thermoregulation) studies in rats are available

Experimental rats were obtained from the Central Animal House Facility, JNU. Rats were maintained under 12:12 h light: dark cycle with food and water ad lib and were kept in standard polyethylene cages with stainless steel lids.

2. **INSTRUMENTATION AND RECORDING SETUP:**

2.1 Stereotaxic Apparatus:

Stereotaxic surgical procedure for the implantation of electrodes (recording and stimulating) involves the use of a stereotaxic instrument (M/S INCO Pvt. Ltd., Ambala, India). By means of a rigid metal frame, the head of the rat was fixed in position using standard skull landmarks. This instrument made it possible to direct an electrode, with three-dimensional control, into local points in the depths of the brain with considerable accuracy.

Stereotaxic Apparatus is an instrument by which any of the brain areas can be approached precisely without major surgical operation and with lesser damage to
other structures of the brain. Such instrument was first designed by Clark and Horsely (1906) and then modified by several other workers. This instrument is based on a 3-dimensional system of coordinates- three planes perpendicular to each other in space, which are referred to external points on the animal skull. The fronto-

![Diagram of brain structures with labels Bregma, Lambda, Incisor teeth, Instrumental Zero, Animal Zero, and 5 mm](image)

frontal plane passed through the centers of the external auditory meati (lateral coordinate), and the horizontal (baseline) plane through the same centers and lower borders of the orbits (vertical coordinate), and the sagittal plane through the midplane of the skull (anterior-posterior distance coordinate). The point where these three planes intersected was arbitrarily considered the zero point and in rats it was 5-mm above the basal plane (horizontal zero).

The instrument is zeroed by bringing the external auditory meatus plugs together so that their tips touch in the midline. The electrode is set in its carrier at a 90° angle from the horizontal plane and moved until its tip touches the point of contact between the tips of the external auditory meatus plugs. The reading on the
electrode carrier thus obtained represents the "instrumental zero." In the rat, an anterior reference point is established rostral to instrument zero. This reference point is selected arbitrarily by using the intersection of an imaginary line drawn between the eyes, which intersects the mid-sagittal suture line. This point of intersection is called the "anterior reference point" or Bregma. The anterior reference point should be exactly 5 mm above instrumental zero; this can be accomplished by raising or lowering the nose-holder and thus pivoting the rat’s head on the meatus plug.

### Material and Methods

#### 2.2 Electrodes:

<table>
<thead>
<tr>
<th><strong>EEG &amp; Ground Electrodes:</strong></th>
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<tr>
<td>Stainless steel plate electrodes were used as EEG and ground electrodes. These electrodes were prepared by soldering approx. 5 cm flexible radio wires onto the small plate screws (Fig 2.3).</td>
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<tr>
<th><strong>EOG &amp; EMG Electrodes:</strong></th>
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<tr>
<td>Flexible radio wires covered by a protective plastic sheath were used as EOG and EMG electrodes. These were prepared by stripping the insulated covering of a flexible wire at the tip. A ring or loop (as shown in the figure) was made at the stripped end and was used as EOG and EMG electrodes respectively (Fig 2.4).</td>
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<th><strong>Stimulating Electrodes:</strong></th>
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<td>Bipolar stimulating electrodes were prepared from epoxy coated stainless steel wires. Two such wires with a diameter of 80-100μm each and 20mm long were glued together along their length. The tip distance was kept maxim. 1mm (Fig 2.5).</td>
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Fig. 2.3

Fig 2.4

Fig 2.5
Material and Methods

Chemitrode:
The bilateral chemitrodes were made by soldering two stainless steel tubings (2 cm long and 24 G diameter) 0.8 to 1.0 mm apart. For each guide cannulae, respective blocker, was made by using 28 G stainless steel wire along with a stopper arrangement on one end so that it can not protrude out from the guide cannula into the brain (Fig.2.6).

Fig. 2.6

2.3 Polygraph:
It is an instrument used for simultaneous recording of multiple biological signals on chart paper. To record the electrophysiological parameters: EEG, EOG, EMG and neuronal activities, 8 channel Grass Polygraph (Model 7H) and 6 channel Lectromed (Model MT8P) polygraphs were used in this study. A polygraph consists of the following components:

1. Writer unit (speed regulator): The main components of the writer unit are the galvanometers, the pens, and the paper-drive mechanism. The coil of each galvanometer is connected through a system of levers to the corresponding pen or stylus, the system being so arranged that an upward deflection indicates a relative negativity of the first input in respect to the second input. Paper speed can be set at speeds of 1mm, 2.5mm, 5mm, 10mm, 50 mm and 100mm/sec or mm/min by pressing respective push buttons. The speed of the paper in this study was set at 2.5mm/sec. Speed of 5mm or 10mm/sec was also used occasionally since faster speed is helpful in defining the exact dimensions and inter-real phase relationships of low and high frequency components and rapid transients.
2. **Time event marker**: It gives tiny marks of increasing amplitudes every 1, 5 and 10 sec. The amplitude of marks every 1 sec, for instance are smaller in amplitude, those every 5 sec are medium and the largest occur every 10 sec. The stimulus marker gave upward or downward deflection depending on the selector. Besides this, the mark could be given with a remote control or with the help of a switch on the body of the Polygraph.

3. **Input**: The signal from the preparation was fed to an 'Electrode board'. The 'Electrode board' was connected to the 'Electrode selector board' attached to the main frame of the Polygraph through a long well shielded cable. The 'Electrode selector board' had G1 and G2 selector switch panel for each of the 8 channels which enabled the selection of positions between 1-24 of the 'Electrode board' to select for selective recording. The selected channel from the 'Electrode selector board' could be connected to respective channels of the main frame of the polygraph for recording. Besides, the 'Electrode board' also had a calibration switch that was common for all the channels and could give voltage varying between 5μV to 20mV in 12 steps of 5-10-20.

4. **Polygraph channels**: Each channel consists of the power switch ON/OFF; indicator light bulb; external input; the differential AC preamplifier and the Driver amplifier.

5. **Differential AC preamplifier**: It has the following specifications:

   (a) **Calibration Switch**: It selected an internal calibration signal which ranged from 10 to 500 on the μV scale and from 1 to 50 on the mV range. The INPUT switch was used to select between the CAL and USE mode. G1 NEG red push button, which when pressed, delivered the calibration signal which deflected the recording pen in the upward direction (negative) and when released, a downward (positive) signal was obtained. The vertical height of signal in either direction was the deflection for the calibration voltage selected at the amplification settings used. Once the calibration was done, the USE mode was selected for recording.
(b) **Sensitivity Switch**: The amplification of the preamplifier could be selected by an adjustable 12-position switch either in 1 to 75μV/mm range (adjust switch pushed upward in μV/mm position) or in the range of 1 to 75mV/cm (adjust switch pushed downward in mV/cm position).

(c) **Frequency Control Response**: It was used to adjust low frequency response. The half amplitude low frequency switch selected one of the 9 cut-off filters ranging from 0.01, 0.1, 0.3, 1.0, 3.0, 10, 30, 100 or 300 Hz.

6. **Driver amplifier**: It further amplified the power of the signal amplified by the preamplifier, proportionate to the signal, to such a level that the pen could be moved. It consisted of the following adjustment knobs:

(a) **Polarity knob**: This knob was used to adjust the movement of the pen, either upward or downward with respect to the baseline.

(b) **Baseline knob**: It enabled the adjustment of the pen along a horizontal line and thus neutralised stray DC, if any.

(c) **50Hz filter**: It selectively rejected waves of line frequency, 50Hz in this situation.

(d) **High frequency cut-off knob**: Depending on selection, it filtered off waves of frequency beyond 30, 10, 3, 1, 0.3, 0.1, 0.03 kHz from the signal.

(e) **Driver sensitivity**: This knob provided continuous adjustment of sensitivity of pen deflection.

The selection of different knobs for recording EEG, EOG and EMG waves in this study were as follows:
2.4 AMPLIFYING AND PROCESSING EQUIPMENTS:

1. AC differential amplifier: AM systems AC differential amplifier (model 1700) was used to amplify the signals from single unit. It had the provision to select between 100, 1000 or 10,000 times amplification with the help of a switch. In this study the signal was amplified 10,000 times by this instrument. It had the facility to limit the frequency ranges of the signal by attenuating the low frequency components in steps of 0.1, 1.0, 10, 100, 300 Hz and the high frequency component in steps of 500, 1K, 5K, 10K, 20K Hz. In this study, the low frequency was filtered at 300 Hz and the high frequency at 5KHz. The differential amplifier has an inverting (-) and a non-inverting (+) input and a single output referenced to the circuit ground. The value of the output depends on the difference between the two inputs.

2. Input impedance: On the input side of an ideal differential amplifier, it is important to assume that the amplifier does not load down the source. The input impedance of the amplifier forms a voltage divider with the output impedance of the source. If all the source voltage is to be applied to the inputs of the differential amplifier, and none lost across the source output impedance, then the amplifier input impedance must be arbitrarily large compared its output impedance. It is also important that all of the output signal should be applied to the load. However, the output impedance of the amplifier forms a
voltage divider with the load, hence the output impedance must be low or zero. The differential amplifier used (AM systems, USA) in this study had a high input impedance (10-100MΩ) and a low output impedance (less than 1KΩ).

3. Common mode rejection ratio: With differential amplification, electrical activity from a pair of electrodes connected to the amplifier was compared and only potential differences between two are registered. Thus any potential, which is picked up equally in voltage and phase at both electrodes (common mode signals), is cancelled out. The effectiveness with which a differential amplifier rejects in-phase signals compared to its ability to amplify out-of-phase signals is called the common mode rejection ratio. The amplifier used in this study had common mode rejection ratios of the order of 10,000:1 at 60 Hz.

4. Gain and sensitivity: The gain of an amplifier refers to its ability to enhance the signal. It is usually expressed as the ratio of the input voltage to the output voltage. It is commonly of the order of 100,000:1 (100dB). Amplifiers have adjustable gain controls, sometimes-called attenuators, which alter the degree of amplification in fixed steps (e.g., 6dB steps will halve or double the input voltage). The term sensitivity refers to the actual voltage relationship between the amplifier input and output. The sensitivity control of the amplifiers was usually continuously adjustable to permit fine setting and equalization of the gain between different amplifiers being used together. Amplifiers must be operated well within their linear amplifying range.

5. Frequency response: It is not only essential to ensure faithful amplification of bioelectrical activity, it is also vital to be able to discriminate the evoked potential from other undesired activities which normally remain inevitably intermixed with the signals. The unwanted activity (noise) originates both from the surrounding and from the electrical devices in the immediate neighborhood of the recording equipment. Thus, the aim in recording was to ensure a large, clear response with the least possible contamination by noise (i.e., an optimal signal-to-noise ratio). Intrusive noise can be reduced by selectively attenuating undesired
frequencies. The range of frequencies that was freely amplified is called the band-pass or bandwidth and this was limited by cut-off points at the low and high frequency ends.

2.5 Oscilloscope:

The oscilloscope is a graph-displaying device. It draws a graph of an electrical signal. In most applications, the graph shows how signals change over time: the vertical (Y) axis represents voltage and the horizontal (X) axis represents time. Oscilloscopes are used by everyone from television repair technicians to physicists. They are indispensable for anyone designing or repairing electronic equipment. The usefulness of an oscilloscope is not limited to the world of electronics. With the proper transducer, an oscilloscope can measure all kinds of phenomena. A transducer is a device that creates an electrical signal in response to physical stimuli, such as sound, mechanical stress, pressure, light, or heat. An automotive engineer uses an oscilloscope to measure engine vibrations. A medical researcher uses an oscilloscope to measure brain waves.

Oscilloscopes also come in analog and digital types. An analog oscilloscope works by directly applying a voltage being measured to an electron beam moving across the oscilloscope screen. The voltage deflects the beam up and down proportionally, tracing the waveform on the screen. This gives an immediate picture of the waveform. In contrast, a digital oscilloscope samples the waveform and uses an analog-to-digital converter (or ADC) to convert the voltage being measured into digital information. It then uses this digital information to reconstruct the waveform on the screen.

(a) Analog oscilloscopes:

When an oscilloscope probe is connected to the circuit, the voltage signal travels through the probe to the vertical system of the oscilloscope. Depending on the setting of vertical scale (volts/div control), an attenuator reduces the signal
voltage or an amplifier increases the signal voltage. The signal travels directly to the vertical deflection plates of the cathode ray tube (CRT). Voltage applied to these deflection plates causes a glowing dot to move. An electron beam hitting the phosphor inside the CRT creates the glowing dot. A positive voltage causes the dot to move up while a negative voltage causes the dot to move down. The signal also travels to the trigger system to start or trigger a "horizontal sweep." Horizontal sweep is a term referring to the action of the horizontal system causing the glowing dot to move across the screen. Triggering the horizontal system causes the horizontal time base to move the glowing dot across the screen from left to right within a specific time interval. Many sweeps in rapid sequence cause the movement of the glowing dot to blend into a solid line. At higher speeds, the dot may sweep across the screen up to 500,000 times each second. Together, the horizontal sweeping action and the vertical deflection action traces a graph of the signal on the screen. The trigger is necessary to stabilize a repeating signal. It ensures that the sweep begins at the same point of a repeating signal, resulting in a clear picture. In conclusion, to use an analog oscilloscope, it is needed to adjust three basic settings to accommodate an incoming signal:

1. The attenuation or amplification of the signal.
2. The time base.
3. The triggering of the oscilloscope.

(b) Digital oscilloscopes:

Digital oscilloscopes contain additional data processing systems. The digital oscilloscope collects data for the entire waveform and then displays it. When a digital oscilloscope probe is attached to the circuit, the vertical system adjusts the amplitude of the signal, just as in the analog oscilloscope. The analog-to-digital converter (ADC), in the acquisition system samples the signal at discrete points in time and converts the signal's voltage at these points to digital values called sample points. The horizontal system's sample clock determines how often
the ADC takes a sample. The rate at which the clock "ticks" is called the sample rate and is measured in samples per second. The sample points from the ADC are stored in memory as waveform points. More than one sample point may make up one waveform point. Together, the waveform points make up one waveform record. The number of waveform points used to make a waveform record is called the record length. The trigger system determines the start and stop points of the record. The display receives these record points after being stored in memory.

A digital storage oscilloscope can save and display signals on the screen. Each signal can either be erased with each new signal or can be stored as a reference signal for comparison. A stored signal can be printed out on a printer attached to the scope. This is one of the advantages over the traditional analog oscilloscope where signals are transient and must be photographed instantaneously to keep a record.

2.6 Spike Discrimination Technique:

The isolation of single units for observation depends on the consistency of the potential's amplitude and shape. Due to active movement or jerk, there are possibilities that the configuration of the neuronal spikes may change. Hence, to record a well-discriminated single unit (signal to noise ratio 3:1) of same shape and amplitude, a window discriminator was used in this study. A window discriminator separates the spike potential from the background biological noise.

For every waveform peak that appears within the set window aperture, a rectangular pulse is generated at the "Within Window" output. Signals exceeding the upper level of the window produce outputs at the "Above Window" output. Visual indication of the pulses is provided at each output by LED. Viewing the input signal as well as the "window" discriminator levels simultaneously provides convenient visualization and ease in setting up an experiment. The lower level control sets the lower discrimination level, and the window aperture control sets the upper window level with respect to the lower level setting. This means that
changing the lower level setting has no effect on the window aperture except to
displace the window up or down. The pulses from either output (Within Window
or Above Window) are generated by the falling edge of the analyzed signal as it
crosses the preset levels.

2.7 Pulse Counter:
The pulse-monitor divider is a signal monitoring and dividing system. Signals (as pulse output) from the window discriminator were fed to pulse counter
circuit, which gave output of pulse for 1:1, 1:2, 1:5, 1:10 & 1:20 pulses. This
helped in easy counting of neuronal activity with faster firing rates for analysis
purposes. The output pulses were of fixed duration of 10msec and were transistor-
transistor logic (TTL) compatible for further recording and processing. The output
pulses had visual as well as audio output and were also recorded in one of the
channels of the polygraph for further analysis. In addition, audio output connected
to the speaker allowed for the continuous and simultaneous listening to the firing
of neurons (action potential), instead of constantly watching on the oscilloscope
screen to see if a cell is active.

2.8 Data Processing Device:
Neural signals are small electrical and chemical events. Sophisticated
equipment are required to record and analyze these events. In this study personal
computers with Pentium III processors (700 MHz), large memory 64Mb SDRAM,
large hard disk (20 Gb storage capacity), excellent graphics program and idle
printer were used for efficient on-line data recording. For off-line data
interpretation, analysis and presentation, Windows 95 based software Spike 2
program (CED, UK) was used.

The computer systematically sampled and stored the input waveform,
adding together a number of individual responses in memory. The continuous
analogue data was digitized, i.e., sampled, measured and converted to a series of
binary numbers suitable for manipulation by computer. This process of analogue-to-digital (A-D) conversion needed a fast sampling rate and a fine voltage resolution to represent the waveform of the input data accurately.

2.9 Analogue to Digital Converter (ADC):

CED-1401 plus A-D converter system was used in this study due to its simplicity of design and flexibility of operation. It is a 16-channel system having sample rates as high as 100 kHz with 12-bit accuracy. The resolution of A-D converter is characterized both by the number of sample points it provides to represent an analysis period for each sample point, and the number of bits used to represent the amplitude. At the very least, the sampling rate of the analog input provided is not less than two-and-a-half sampling points to define the period of the fastest frequency.

The amplitude of the evoked potential is digitally reproduced, which depends on the number of bits per sample in the computer’s analogue-to-digital converter. This fixes the number of distinguishable voltage levels, which can be recorded and is likely to be eight bits or more. An eight-bit A-D converter will resolve a specified input voltage range into 256 discrete levels. This is satisfactory if the range of input voltages is well matched to the voltage range of the A-D converter.

After sampling and conversion of the sampled elements of the signal to quantized form (numbers), the data was stored for analysis. The stored signal was transformed into a new form known as the frequency or Fourier transformed (FFT) signal. The process may be thought of as the filtering of the stored signal by a set of band-pass filters whose outputs were accumulated during the passage of the signal through the filters. Each accumulated filter output then becomes a point in a new output plot or curve.
2.10 Stimulator:

The stimulator S44 (Grass Instrument Co. USA) was used. The stimulator generates uni-directional square wave pulses having varying voltages, duration and frequencies. Stimulus intensities of the pulse output could be varied between 10mV to 150V, pulse duration from 10 μs to 10 sec and frequencies 1/100 sec to 1000 PPS. Each output pulse could be adjusted with a delay from 10 μs to 10 sec.

In addition, there is a trigger output, which was used to trigger the oscilloscope beam while recording.

2.11 Stimulus Isolation Unit:

Direct electrical stimulation of nerves for the derivation of somato-sensory potential gives rise to large electrical transients that may obscure the response or cause momentary improper operation of the amplifiers. These effects result from the conduction of currents created by the electrical stimulus through the body to recording and ground leads. To reduce these effects, it was desirable to confine these currents to the area stimulated, as much as possible. This requires isolation of the current source from the ground and may be accomplished by a stimulus isolation unit, which provides a stimulus that is, in effect, disconnected from the input signal and the system ground.

In this study photoelectric stimulus isolation unit PSIU6, Grass, USA was used. It is an optically isolated constant current unit, which provide current from 0.1μA to 10mA. It has a light emitting diode, driven directly by the output voltage.
of the stimulator (square wave output), which was fed to this device (instrument). This input caused a current to flow through the photoelectric diode to the stimulus (voltage) which appeared as an input signal to the built in constant current transistor. Output current was controlled by current range switch and the stimulator volt dial was taken from the collector circuit of the constant current transistor.

PSIU6 comprises of the following components:

(a) **Polarity Switch**: PSIU6 had a toggle switch to change the polarity of the pulses. In ‘Normal’ position of the switch the red output terminal was positive with respect to the black output terminal. In the ‘Reverse’ position the black output terminal was positive with respect to the red terminal.

(b) **Current Range**: The current range could be selected by a 5-position current selector knob. The ranges were 0.1 μA - 1.5μA; 1.0 μA - 15μA; 10μA - 150μA; 0.1mA - 1.5mA and 1.0mA - 15mA.

(c) **Multiplier Switch**: PSIU6 had output impedance of 2000 Ω. The instrument driving this isolation unit should have output impedance 5-10 times less than that of the PSIU6 input impedance (i.e., 200-400 Ω). On the other hand, a driving voltage less than 10 volts would not supply sufficient input to the light source of PSIU6 to provide for the meaningful output current. Therefore to match these two conditions, the output multiplier switch of the S44 stimulator was kept at X10 (SIU) during use.

2.12 Microiontophoresis:

(a) **Basic Principle**:

In the simplest case, each barrel of a micropipette assembly to be used for drug ejection is filled with a solution of ionized compound and the solution connected to the iontophoretic instrument by a suitable lead. As for other
electrophysiological situations, the business end of the lead which is in contact with the drug solutions should be of the non-polarizing variety, i.e. platinum or silver/silver chloride. The establishment of a potential difference between the drug solution and the medium surrounding the barrel tip will cause movement of ions through the solution and out of the pipette tip. An outward current will cause 'ejection' of positively charged ions, an inward current ejection of negatively charged particles. A drug molecule tends to diffuse from the solution through the pipette tip to the extra cellular environment. The efflux of drug molecule can be checked or reduced by applying a small current that is known as a holding, backing, braking or retaining current. It is also in usual practice to include a barrel containing NaCl or KCl solution, which can be used as a control for the effects of current itself. This may be done either by periodically passing through the control barrel the same current used for drug ejection or by passing continuously a current adequate to cancel out the instantaneous sum of ejecting and retaining currents.

Fig. 2.7(A): Microiontophoresis proper; ion movements result directly from the application of a potential difference between the inside and outside of the pipette barrel Fig. 2.7(B): Electro-Osmosis; this usually contributes to the total release resulting from an applied voltage. It is due to the existence of an electrical 'double layer' at a glass/water interface, giving the solution a net positive charge. A volume of solution, with dissolved drug molecules (Z) is, therefore ejected by outward current.
passing through the drug-containing barrels. This is known as current balancing. In the years since Nastuk, 1953 reported results with his electrically controlled microjet, microiontophoresis has been used to study systems ranging from single neurons and glia in culture (Hosli and Hosli, 1978) to the process of thrombus formation in blood vessels (Begent and Born, 1970). To date, microiontophoresis remains an enormously valuable, flexible and informative technique.

In this study, S7061A Microiontophoretic Instrument from WPI, USA has been used.

(b) Electrode Assemblies for Microiontophoresis:

Microelectrodes and microiontophoretic assemblies can be readily manufactured from borosilicate glass tubing. For the commonly used five- or seven barreled multibarrel assemblies several pieces of tubing are fused together before pulling. The appropriate number of tubes are fastened together at their ends by a strong (heat-resistance) adhesive, a metal collar, heat-shrink tubing etc. and the top and bottom of this electrode ‘blank’ are then held by chucks in the electrode puller. A heating coil is used to melt the glass gently in the central portion of the assembly and the lower chuck is then rotated slowly by hand while exerting a small amount of pull on the glass. This rotation and slight pulling cause the lengths of tubing to fuse together. The coil is now switched off and the tubing is allowed to cool.

It probably does not matter greatly which of the various types of capillary tubing available is used in the manufacture of multibarrel blanks for extracellular use; it is more important if intracellular microiontophoresis or pressure ejection is to be used. For example, the use of thin-walled capillaries will produce pipettes with longer shanks and smaller external tip size for a given resistance than the more usual capillary tubing and these would clearly be preferable for intracellular penetration.
(c) Puller:

Many types of electrode pullers are available which are suitable for pulling multibarrel assemblies. Some pull vertically, some horizontally; the pulling force may be mechanical, gravitational or electromagnetic. While any of the puller is adequate to produce multibarrels for extracellular use, the main difference between the vertical and horizontal puller is that in the former the lower chuck is exerting pull on the tube, as it begins to soften, due to gravity. In most horizontal pullers this initial phase of pull can be more readily controlled electromagnetically. In the Livingston Puller no tension is exerted on the tube until the glass has been heated to the required fluidity. The full extent of a preset force is then applied to a single pull. The amount of heat applied and the delay to pull can be varied.

(d) The Pulling Process:

There is a great deal of art involved in the pulling of electrodes and especially multibarrel blanks. This is simply because the size of a multibarrel blank is such that the diameter of the platinum heating coil, can have a major effect on heat distribution to the glass. The combination of heat and pull may be varied to produce pipettes of different tips lengths and tip diameters. For getting most consistent results, it is necessary to reset the puller when a new batch of capillaries or blanks is obtained.

(e) Filling:

The method chosen for filling the micropipette barrels will depend mainly on the type of micropipette. The simplest to fill are those in which a glass filament has been fused along the inside wall of each barrel of the blank, the so called omega-dot (Hare) tubing. The junction between the filament and tubing wall causes the movement of any liquid placed anywhere in the tube to be transported along the length of the tube by capillarity. Because of the fineness of the barrel tip and its taper, fluid is carried most rapidly into the tip. As more fluid coats the
internal surface of the tip, the surface tension of the water causes the solution to occupy the whole cross-section of tube at the extreme tip. This then draws more fluid down the tube along the glass filament due to surface tension and the pipette fills. In this study Kwik Fil Glass Capillary from WPI, USA has been used and those were of the omega-dot variety. A hypodermic syringe and needle with a fine flexible length of polyethylene tubing attached is adequate for placing solution into the larger pipettes which have been used in this study. However, 30 gauge Luer fitting syringe needle is also available from WPI and Shrimpton and Fletcher.

(f) Drug Solutions:
All solutions should be made in at least double distilled water and preferably deionized water, due to the facts that the presence of small highly mobile ions may result in their carrying a significant fraction of the iontophoretic current, especially if the compound of interest is of high molecular weight or is poorly ionized. The term 'osmotic artifact' is used to refer to the bulk flow of solution, carrying the active ion, which could result if the solution in the pipette were present at a substantially lower osmotic concentration than the biological fluids with which the tip is in contact. To minimize this, it is usual, whenever possible, to use ionized compounds at a concentration of 150-200mM. The pH of drug solutions is often adjusted either to increase solubility or to optimize the degree of ionization of weak acids and bases.

(g) The Assessment of Electrodes:
The careful microscopic examination of an electrode tip may satisfy the observer as to its general condition, size and profile of the tip. Exposed tip area (tip diameter) and other features of the tip determine the resistance of microelectrodes as it known that $\Omega \propto 1/r^2$. In our study the recording barrel had a tip diameter of about 2-3 $\mu$m and resistance of about 27 ± 0.07 M$\Omega$. 

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3. **ANIMAL AND SURGICAL PROCEDURES:**

3.1 Pre-operative care:

Experiments were performed on male wistar rats weighing between 250-300 gm. The animals were housed in a 22-24 °C temperature controlled room on a 12:12 h light: dark photoperiod. They had access to food and water *ad libitum*. The surgery as well as recording rooms were regularly cleaned.

(a) **Anaesthesia**:

1. **For Single Unit recording**:

As the single unit recording was to be done in anaesthetized & head restrained condition, therefore, each animal was anaesthetized with intraperitoneal injection of long lasting light anaesthesia; urethane (Sigma) (1.0g/kg body weight). The anesthetic effect of the urethane injection lasted for the duration of experiment. Xylocaine, a local anaesthetic was applied at the orifice of the rectum at the time of rectal probe insertion into the rectum (2.0-3.0 cm).

2. **For Free Moving animal**:

The surgical procedure for electrophysiological parameters recording in free moving animal lasted for 2-3 hrs, therefore, short lasting deep anaesthesia sodium pentabarbitone (Sigma) was used. Each animal was given intraperitoneal injection of 35mg/kg (i.p.) sodium pentabarbitone and the animals were prepared for chronic recording of bipolar electroencephalogram (EEG), electroocculogram (EOG) and electromyogram (EMG).

(b) **Surgery**:

1. **For Single Unit Recording**:

The hair from the head of anaesthetized male wistar rat were cut and the animal was placed in a stereotaxic apparatus (INCO, India). An incision was given on the midline over the skin on the skull to deflect the skin aside and the exposed
skull was cleaned. A window of approximately 4mm diameter was made on the midline 0.5 mm behind the Bregma so that the mPOAH (AP- 0.5; L- 0.5; H- 8.0) could be accessed by glass microelectrodes. Two EEG electrodes were implanted bilaterally at stereotaxic coordinates A + 2.0, L 2.0 to record fronto-occipital EEG and a third one was fixed on the midline over the frontal sinus, which served as a reference electrode. A bipolar stimulating electrode was implanted unilaterally into the mid brain reticular formation (MRF) at co-ordinates A-6.0, L-1.5, H-7.5 mm. One stainless steel cannula (1.0 mm in diameter) was implanted angularly at the coordinate of AP +2.0mm, L 0.5mm and H 7.5mm, so that a copper-constantan semi micro-thermocouple (less than 0.5 mm diameter) could be inserted through it to record the brain temperature (Tbr) near the mPOAH. All the electrodes and steel tubings were fixed on the skull with the help of acrylic cement (Ashwin, India). The exposed brain tissue was covered by wax.

2 For S-W recording in freely moving animal:

The animal was shaved and placed in a stereotaxic instrument. With the help of a scalpel, a midline incision was made, the skin was deflected, the top of the skull was scraped and the skull surface was thoroughly cleaned with normal saline. Two screw electrodes were bilaterally implanted on the skull for recording EEG and a third one was implanted on the midline to provide animal ground. A pair of flexible insulated wires (except at the tips) were connected in the dorsal cervical neck muscles to record EMG and another pair of wires was used to record EOG from the external canthus. The free ends of all the electrodes were connected to a 9 - pin female plug, which in turn was fixed to the skull of the animal with dental acrylic. Bilateral chemitrode, made up of 24 - gauge stainless-steel tubing (guide cannulae) along with respective blockers, were implanted through drill holes made in the skull at stereotaxic coordinates A -0.3 to -0.8 mm, L 0.6 to 1.2 mm to microinject chemicals into the mPOAH. The chemitrode was fixed on the skull with dental acrylic once its tip reached H 6.0 mm. The injector made from 30-gauge
stainless-steel tubing had a stopper arrangement so that it would protrude 2mm beyond the guide cannula to reach mPOAH. A copper-constantan thermocouple was implanted at an angle so as to record brain temperature near the mPOAH.

3.2 Post-Operative Medication and Care:

The animal was observed and cared for during its recovery from the surgical trauma. The wound was regularly dressed and Nebasulf sprinkling powder (Pfizer, India) was applied until the wound had completely healed. At least 4 days were allowed for recovery from the surgical trauma and wound healing.

3.3 Acclimatization to the Recording Chamber:

During its recovery from surgical trauma, day 2 onwards the animal were kept in the recording chamber for at least 3-4 hrs to get them acclimatized to the environment of the recording setup.

4. PROCEDURE FOR RECORDING AND DATA ACQUISITION:

4.1 Experimental Protocol for Single Neuronal Recording:

After recovery from surgical trauma, few cycles of sleep-wakefulness were recorded and immediately after that the rat was anaesthetized and placed in the stereotaxic apparatus. The animal was placed on a platform with an arrangement for conductive heating or cooling (by passing warm or cold water as the case may be) so that the body temperature of the rats could be increased or decreased. A copper-constantan semi micro-thermocouple was fitted in the cannula to record brain temperature near the mPOAH. An additional thermocouple was inserted 2.0-3.0 cm into the rectum to monitor core temperature. The rectal (Trec) and brain (Tbr) temperatures were manipulated up to ± 3°C by changing the platform temperature. The exposed part of the brain was covered with paraffin oil.

Three-barrel glass microelectrodes with a resistance of 20-30 MΩ and 5-8
μm tip size were pulled afresh by using the Narishige horizontal puller as described above. One of the barrels was used as recording barrel and was filled with the 2% pontamine sky blue dissolved in 0.5 M sodium acetate. Rest two barrels were used as injecting barrels. In one of the injecting barrels 3M KCl was filled and used as control barrel while in another injecting barrel either 2mM-prazosin, an α-1 adrenoreceptor antagonist or 5mM picrotoxin, a GABA-A receptor antagonist (depending on the experiments) was filled, respectively. The electrode was stereotaxically advanced slowly in free run condition through the brain by using remote control hydraulic microdrive until it reached the upper boundary of mPOAH. Thereafter, it was advanced in steps of 2-3 μm until a well discriminated single unit (signal-to-noise ratio at least 3:1) was encountered. The signal was passed through WPI high-impedance probe connected to WPI S 7200 HV module, amplified by A-M System preamplifier and fed into a window discriminator (FHC, USA). The raw signal was displayed on a TDS210 (Tektronix) oscilloscope and fed to a computer through an ADC (CED 1401) interface. The processed output of the single unit activity from the window discriminator was recorded on a polygraph paper.

The thermosensitivity of a neuron was ascertained by correlating its firing rate with changes in Trec. Simultaneous change in Tbr was also noted. After that, either prazosin or picrotoxin was injected iontophoretically by passing +ve 75 nA or +ve 80 nA current respectively, through WPI M 701 Micro Probe Current Injector system for 60 sec and the effect on the neuronal activity was recorded. The effect of current was studied by injecting the same amount of current through the other KCl filled barrel. The microelectrode was then advanced until another stable single unit was encountered.

4.2 MRF Stimulation Induced EEG Desynchronization (100Hz):

(a) In Free Moving Animal:

High frequency (100Hz, 200μs, 150-250 μA) stimulation for short duration (8-10sec) was delivered to the wake inducing region in the rostral brainstem, the
MRF. This stimulation strength was found sufficient to induce cortical EEG desynchronization and wakefulness. The stimulation induced desynchronized EEG outlasted the period of stimulation. The significance of MRF stimulation in freely moving animals to induce cortical EEG desynchronization was (a) to confirm the proper site that induces EEG desynchronization (b) to study the effect of anesthesia if any and (c) the visualization of the adverse effect if any, on the animal's behavior due to the stimulation strength used in this experiment.

(a) *In Urethane Anaesthetized Animal:*

The strength of stimulation, was similar as mentioned earlier, was sufficient to induce EEG desynchronization in urethane anaesthetized animal. The induced cortical desynchronization lasted for at least 40-50 sec.
4.3 Effects of 1Hz Stimulation of MRF on the Neurons:

In order to study if the mPOAH neurons received any projection from the MRF (wake area) stimulation site, the response of the neurons to 1Hz (200μs, 500-600μA) stimulation of MRF was studied by overlapping 10 stimulus bound responses on the oscilloscope (TDS 210).

4.4 Experimental Protocol for recording Sleep-Wakefulness and Body Temperature:

Recording Before and After Picrotoxin Injection:

After recovery, bipolar EEG, EMG and EOG were recorded in three separate channels of a Grass polygraph. The rectal and brain temperatures were recorded every 10 min. The experiments were conducted between 10 am-7 pm. The same animal served as its own control where recording was conducted without injection in normal condition (baseline), after bilateral saline injection (control) and after bilateral picrotoxin injection (experimental) into the mPOAH. Baseline recording was done on day 1 followed by control that is recording after saline injection on day 2. After a gap of one day recording was done on day 4 after picrotoxin injection into the mPOAH. In control and experimental groups after normal recording of 1 h, 250 nl of either saline or 0.1% (250 ng in 250nl) picrotoxin,
respectively was locally microinjected bilaterally into the mPOAH at a rate of 100 nl/min. The injector was retained in the same position for at least one-min after injection and then replaced by the blocker. The injection procedure took 7 - 8 min and no recording could be done during that period. After injection the same parameters were recorded continuously for eight hours.

4.5 Histological Verification:

(a) Identification of Neuronal Recording and Stimulation Sites:

At the end of recording -ve 20μA current was passed through the barrel containing pontamine sky blue for 15 min to deposit the dye at the last recording site which was then identified histologically. At the stimulation site electrolytic lesion was made by passing anodal direct current 500 μA for 20 sec (Grass DC Lesion Maker). The animal was then perfused intracardially with normal saline followed by 10% formaldehyde containing 2% potassium ferrocyanide. Because of electrolytic lesion, ions of iron were deposited at the site of stimulation, which gave Prussian blue coloration due to the formation of potassium ferro-ferricyanide complex. The brain was removed and kept in 10% formaldehyde for 24 hrs followed by 30% sucrose solution. Thereafter, 40 μm thick sections were cut and stained with 2% neutral red. The recording sites were identified from the histological sections by the presence of tip of the electrode track and presence of blue spot.

(b) Identification of Microinjection Sites:

After the experiments, under deep anaesthesia (Pentobarbitone Sodium 35 mg/kg) the rats were injected with 250 nl (same volume as that of the injected chemicals) pontamine sky blue (2% solution) into the mPOAH in the same manner as the drug. After 20-30 mins. the animals were intracardially perfused and histological sections were obtained by following the same procedures as mentioned above.
The brain samples were kept in 30% sucrose solution until the brain sank. The purpose was to remove all traces of the fixative from the tissue and to provide cryoprotection. At the time of sectioning, the sample was taken out from the sucrose solution and then embedded in an OCT compound (Tissue-Tek, Miles Inc. USA). The OCT compound firmly holds the frozen specimen onto the cryostat chuck or the block holder, providing more stability and protecting the tissue from sudden impact with the knife's cutting edge. The sectioning of frozen brain tissue was performed on a Cryostat (Leica, Germany). 40 μm thick sections were cut on a cryostat. They were collected directly on subbed slides and air dried at room temperature for at least 1 hour before staining. The site of recording was identified by the presence of blue spot.

### Table 2.3: SUBBING AND STAINING OF HISTOLOGICAL SLIDES

<table>
<thead>
<tr>
<th>Subbing Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Slides were cleaned with alcohol.</td>
</tr>
<tr>
<td>2. Subbed with a gelatin adhesive solution and dried at room temperature.</td>
</tr>
</tbody>
</table>

**Subbing Solution Preparation:**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin</td>
<td>1.5 g</td>
</tr>
<tr>
<td>30% ethyl alcohol</td>
<td>100 ml</td>
</tr>
<tr>
<td>Glacial Acetic acid</td>
<td>7 ml</td>
</tr>
<tr>
<td>Chrom Alum</td>
<td>0.1g (Chromic potassium sulphate)</td>
</tr>
</tbody>
</table>

- a. Gelatin was dissolved in glacial acetic acid by mild heating.
- b. 30% ethyl alcohol was added.
- c. Chrom Alum dissolved in 2 ml distilled water was added.
DATA ANALYSIS AND STATISTICAL PROCEDURE:

5.1 Classification of Neurons:

Thermosensitive neurons in the mPOAH were classified as WSN and CSN if their percent firing rates $0^\circ C^{-1}$ s$^{-1}$ increased or decreased by $>10\%$ of respective basal rate by increasing or decreasing the temperature respectively. Those neurons whose firing rate did not meet this criterion were termed as temperature insensitive neurons (ISN). In this study, % change/sec/$^\circ C$ criterion has been used to establish the thermosensitivity of the neurons due to the limitations in other criteria as $Q^{10}$ and imp/sec/$^\circ C$. However, thermosensitivity of the neurons was classified by the other methods too i.e. impulse $0^\circ C^{-1}$ s$^{-1}$ and $Q^{10}$ methods. If the neuronal firing rate increased by $>0.8$ impulse $0^\circ C^{-1}$ s$^{-1}$ it was termed as WSN, while if it decreased by $<0.6$ impulse $0^\circ C^{-1}$ s$^{-1}$ it was termed as CSN. In the other method, if the $Q^{10}$ was $>2$ it was termed as WSN and if $Q^{10}$ was $<0.5$ it was termed as CSN. The $Q^{10}$ value were obtained by following formula:

$$\left(\frac{R_1}{R_2}\right)^{10/T_1-T_2}$$

where

- $R_1$ = basal firing rate
- $R_2$ = experimental firing rate
- $T_1$ = basal temperature
- $T_2$ = experimental temperature
In our study, the neurons which met the criterion of 10% according to the percent firing rates $^{\circ}C^{-1} s^{-1}$ change also met the criterion of $Q^{10}$ value.

5.2 Categorization and Statistical Analysis of Unit Firing before and after drug Injection:

Microiontophoretic injections of drugs (Picrotoxin and Prazosin) altered the firing rate of the neurons to different degrees (2.77 % to 418.7 %). However, as mentioned above, the temperature sensitivity of the neurons was decided based on the change in their firing rates by at least 10% per second. Hence, the same criterion was used here also i.e. a drug was considered to increase or decrease the firing rate of the neurons if it was able to increase or decrease the firing rate by more than 10% per second of neuronal pre-injection firing rate. Thus, if the drug-induced change in the firing rate was less than 10% per second, it was considered ineffective on that neuron. Thereafter, to test the level of statistical significance of change in the firing rates of the neuronal activities, ANOVA was applied on those neurons whose firing rate changed by more than 10% on application of drugs.

5.3 Classification of SLEEP-WAKE Stages:

The polygraphic records were first scanned to mark obvious disturbances, if any, which were marked during recording session. The records were then scored in bins of 10sec epoch and subdivided into Active wakefulness (AW), Quiet awake (QA), Slow wave sleep (SWS1), Deep sleep (SWS2) and REM sleep as per the criteria shown in table below (Table 3.5).

The AW was classified by the presence of desynchronized EEG accompanied by high EMG tone and/or muscle movement and eye movements in the EOG. The QW was characterized by the presence of occasional spindling, no active muscle movement and reduced eye movements. The SWS1 was characterized by the presence of EEG synchronization up to 50% of the recording...
Table 2.5

<table>
<thead>
<tr>
<th>STATE OF SLEEP: WAKEFULNESS</th>
<th>EEG</th>
<th>EOG</th>
<th>EMG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ACTIVE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AWAKE (AW)</td>
<td>Desynchronized Voltage ≈ 25-40 μV</td>
<td>Few</td>
<td>High muscle activity</td>
</tr>
<tr>
<td></td>
<td>Frequency ≈ 20-40 Hz</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QUIET AWAKE (QA)</td>
<td>Desynchronized Occasional spindling Voltage ≈ 15-40 μV</td>
<td>Less frequent</td>
<td>Lower muscle tone</td>
</tr>
<tr>
<td></td>
<td>Frequency ≈ 20-40 Hz</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLOW WAVE SLEEP (SWS1)</td>
<td>Synchronized Voltage ≈ 50-200 μV Frequency ≈ 12-15 Hz</td>
<td>Few</td>
<td>Low as compared to QA</td>
</tr>
<tr>
<td>DEEP SLEEP (SWS2)</td>
<td>Synchronized Voltage ≈ 100-200 μV Frequency ≈ 8-10 Hz</td>
<td>Absent</td>
<td>Very low</td>
</tr>
<tr>
<td>RAPID EYE MOVEMENT SLEEP (REMS)</td>
<td>Desynchronized Voltage ≈50 μV Frequency ≈ 20-40 Hz</td>
<td>Frequent monophasic eye movements</td>
<td>Muscle atonia</td>
</tr>
</tbody>
</table>

Time, reduced EMG tone and reduced eye movements. The SWS2 was characterized by the presence of synchronized EEG (>75% time), significantly reduced muscle tone and no eye movements. The REM sleep was scored when muscle atonia and frequent eye movements accompanied EEG desynchronization, usually following a stage of SWS2.

The baseline and saline values of the mean percentage of time spent in AW, QA, SWS1, SWS2 and REM sleep were calculated and compared with those after microinjections of picrotoxin into the mPOAH. The time spent by the rats in these stages before and after picrotoxin microinjection was statistically compared to the respective values after saline injection in different groups by applying ANOVA available from a statistical package SigmaStat.
5.4 Comparison of Brain and Rectal Temperature:

The brain and rectal temperatures were recorded every 10 min. The mean brain and rectal temperatures during pre- and post-picrotoxin injections were compared with those after saline microinjection and baseline values by using the Mann-Whitney test for different groups of rats and Wilcoxon matched-pair signed-rank test for the same group of rats.

6. STATISTICAL ANALYSIS OF THE DATA:

Mean - The mean is the sum of all the scores divided by the number of scores. The mean value can be calculated as:

\[
\text{Mean} = \frac{\sum x}{N} \quad \text{(where } N \text{ is the number of scores)}
\]

Standard Deviation - The standard deviation (SD) quantifies variability or scatter among the values in a column.

Standard Error of Mean - The SEM is calculated as the SD divided by the square root of sample size.

Median - The median is the middle of a distribution or 50th percentile. Half the values are larger than the median, and half are lower. If there are an even number of values, the median is defined as the average of the two middle values.

6.1 Analysis of Variance (ANOVA):

The purpose of analysis of variance was to test the differences in means (for groups or variables) for statistical significance. This was attained by analyzing the variance that is by partitioning the total variance into the component due to true random error and the components which are due to differences between means. These latter variance components are then tested for statistical significance and if significant, we reject the null hypothesis and accept the alternative hypothesis.
according to which the means (in the population) are different from each other. Under the null hypothesis, the variance estimated based on within-group variability should be about the same as the variance due to between-groups variability. These two estimates of variance can be compared via F test, which tells whether the ratio of the two-variance estimates is significantly greater than one.

The probability value 'p', tells if the F is large enough to conclude there is a real (statistically significant) relationship between the variables. If the $p$ is less than 0.05 level (sometimes called the 5% level) or 0.01 level (1% level) it represents a statistically significant relationship between the group tested. The 'p' essentially tells the probability of being wrong in concluding that there is a relationship. As a standard, $p<0.05$ was used in this study suggesting that there may be possible error of 5 or fewer times out of 100. The 0.01 level is more conservative than that of the 0.05 level.

6.2 Student-t Test:
The t statistic is the ratio:

$$ t = \frac{\text{difference of sample means of the two groups}}{\text{standard error of difference of sample means}} $$

The standard error of the difference is a measure of the precision with which this difference can be estimated. It can be concluded from "large" absolute values of $t$ that the samples were drawn from different populations. A large $t$ indicates that the difference between the treatment group means is larger than what would be expected from sampling variability alone (i.e., that the differences between the two groups are statistically significant). A small t (near 0) indicates that there is no significant difference between the samples.

6.3 Mann-Whitney Rank Sum Test:
The Mann-Whitney rank sum test is used to test the null hypothesis that two samples were not drawn from populations with different medians. The Mann-
Whitney rank sum test is a non-parametric procedure, which does not require assuming normality or equal variance. The Mann-Whitney rank sum test is used to find out:

- if the medians of two different samples are significantly different
- if the samples are NOT drawn from normally distributed populations with the same variances, or it is assumed that they were drawn from normal populations.

6.4 Wilcoxon Signed Rank Tests

The Wilcoxon signed rank tests the null hypothesis that two samples were drawn from populations with the same medians. The Wilcoxon signed rank test is also a non-parametric procedure, which does not require assuming normality or equal variance. A Wilcoxon signed rank test should be used:

- if the effect of a single treatment on an individual is significant
- the treatment effects are NOT normally distributed with the same variances

A signed rank test ranks all the observed treatment differences from smallest to largest without regard to sign (based on their absolute value), then (+ve or -ve) sign was attached of each difference to the ranks.

The signed ranks are summed and compared. This procedure uses the size of the treatment effects and the sign. If there is no treatment effect, the positive ranks should be similar to the negative ranks. If the ranks tend to have the same sign, it can be concluded that there was a treatment effect (i.e., that there is a statistically significant difference before and after the treatment).

6.5 Pearson correlation:

Pearson product moment correlation is measured when:
- the strength of association between pairs of variables without regard to which variable is dependent or independent
- the relationship, if any, between the variables is a straight line
- the residuals (distances of the data points from the regression line) are normally distributed with constant variance

When an assumption is made about the dependency of one variable on another, it affects the computation of the regression line. Reversing the assumption of the variable dependencies results in a different regression line. The Pearson product moment correlation coefficient does not require the variables to be assigned as independent and dependent. Instead, only the strength of association is measured. Pearson product moment correlation is a parametric test that assumes the residuals (distances of the data points from the regression line) are normally distributed with constant variance.
RESULTS
In this study the role of GABA-ergic inputs on sleep-wakefulness and body temperature simultaneously as well as the effects of GABA-A receptor antagonist and α-1 adrenoreceptor antagonist on the thermosensitive and insensitive neurons in the mPOAH have been studied. Besides this, the types of inputs from the brainstem to the thermosensitive and insensitive neurons in the mPOAH have also been investigated. Hence, the results of this study comprise of the effects of (1) microinjection of picrotoxin, a GABA-A receptor blocker, into the mPOAH and its effects on sleep-wakefulness and body temperature (2) microiontophoretic injection of picrotoxin, a GABA-A receptor blocker, on the mPOAH neurons (3) microiontophoretic injection of prazosin, an α-1 adrenoreceptor blocker, on the mPOAH neurons, and (4) stimulation of MRF, a wake inducing area on the thermosensitive and sleep-wake related neurons of the mPOAH. The results obtained from this study are grossly discussed in detail under following sub headings for convenience.

► **Picrotoxin Microinjection Study:**

Picrotoxin, a GABA-A receptor blocker, was microinjected into the mPOAH in free moving rats. The effects of microinjection of picrotoxin on sleep-wakefulness and thermoregulation will be discussed as:

(i) Effect on sleep-wakefulness.
(ii) Effect on brain temperature.
(iii) Effect on rectal temperature.
(iv) Correlation between brain and rectal temperature.

► **Microiontophoretic Injection Study:**

(a) Picrotoxin microiontophoretic injection study:

The effects of picrotoxin on different groups of mPOAH neurons will be discussed as:

(i) Effect of picrotoxin on warm sensitive neurons.
(ii) Effect of picrotoxin on cold sensitive neurons.
(iii) Effect of picrotoxin on temperature insensitive neurons.
(b) Prazosin microiontophoresic injection study:
The effects of prazosin on different groups of mPOAH neurons will be discussed as:

(i) Effect of prazosin on warm sensitive neurons.
(ii) Effect of prazosin on cold sensitive neurons.
(iii) Effect of prazosin on temperature insensitive neurons.

Methodological Consideration
There are some methodological limitations in the microiontophoresic study. The primary limitation was the recording of a neuron with the help of glass microelectrode, which is not possible in free moving animals. Therefore, the correlation of neuronal firing with their proper functional behavior is somewhat difficult to establish.

The recording of spontaneous sleep-wakefulness cycle under urethane anaesthetized condition is quite difficult. Therefore, in this study only few EEG related neurons could be recorded.

MRF STIMULATION STUDY:
The MRF area is known to be a wakefulness inducing area. The results obtained after the electrical stimulation of this area will be discussed as:

(a) Effect of High Frequency Stimulation:
(i) The effects of high frequency MRF stimulation (100µA, 100Hz and 200µs) on the cortical EEG in free moving as well as in anaesthetized condition. The effects on the mPOAH thermosensitive and insensitive neurons along with simultaneous changes in cortical EEG. The effects on the putative sleep-wake related neurons.
(b) **Effect of 1Hz Stimulation:**

The effects of 1Hz stimulation have been discussed as:

(i) Effect on warm sensitive neurons.

(ii) Effect on cold sensitive neurons.

(iii) Effect on thermo-insensitive neurons.

(iv) Effect on putative sleep-wake related neurons.

[A] **MICROINJECTION STUDY:**

**Picrotoxin Microinjection into the mPOAH:**

**Effect on sleep and wakefulness:**

An hour wise analysis showed that saline microinjection into the mPOAH did not significantly affect S and W. However, picrotoxin microinjection (0.1%) significantly increased wakefulness (Fig. 3.3) i.e. sleep (deep sleep as well as REM sleep) was significantly reduced (Table 3.1). An hour wise analysis showed that total wakefulness remained significantly increased up to the 5th hour while the increase in active wakefulness was significant only up to the 3rd hour (Table 3.2).

**Effect on brain temperature:**

The mean (± S.E.M.) brain temperature after saline injection into the mPOAH was 38.5 ± 0.03 °C which was not significantly different than that of its mean baseline value of 38.6 ± 0.04 °C (Fig. 3.4 A). However, after picrotoxin infusion the mean brain temperature significantly (p < 0.001) increased to 39.4 ± 0.08 °C (Fig. 3.5) with a maximum of 39.8 ± 0.07 °C which was recorded two hours after picrotoxin infusion. Like the rectal temperature, the significant increase in the brain temperature lasted till 6 hours post-injection (Fig. 3.5).

**Effect on rectal temperature:**

The mean (± S.E.M.) baseline rectal temperature was 37.4 ± 0.04 °C and after saline injection it was 37.5 ± 0.07 °C. Picrotoxin infusion into the mPOAH
significantly increased (p < 0.001) the mean rectal temperature to 39.3 ± 0.05 °C with a maximum of 40.02 ± 0.2 °C which was recorded for more than 3 hours after the injection (Fig. 3.4B). The significant increase in the rectal temperature lasted for more than 6 hours post-injection (Fig. 3.5).

**Correlation of brain and rectal temperatures:**

Comparison of the brain and rectal temperatures in baseline and saline groups of rats showed that the brain temperature was always higher than the rectal temperature (Fig. 3.5). However, in the picrotoxin infused groups, both the brain and the rectal temperatures were elevated and reached a comparable level (Fig. 3.5). The rate of increase in the rectal temperature was higher than that of the rate of increase in the brain temperature (Fig. 3.6a). At the end of second hour post-injection the rectal temperature was higher than that of the brain temperature and it was maintained for the next 3 hours (Fig. 3.5). Pearson correlation test showed that a linear correlation existed between the brain and the rectal temperature. It was observed that picrotoxin induced increase in brain temperature correlated significantly positively (r = 0.994, p < 0.01) with picrotoxin induced increase in the rectal temperature (Fig. 3.6b).

[B] **MICROIONTOPHORETIC INJECTION STUDY:**

(a) **Microiontophoretic Injection of Picrotoxin on the mPOAH Neurons:**

Out of a total of 118 neurons, 63 (53.4%) were thermosensitive while the rest 55 (46.6%) were ISNs. Among the thermosensitive neurons 29 were WSNs, while 34 were CSNs. The group mean (± S.E.M.) firing rate for WSNs was 7.45±0.9 Hz, for CSNs it was 2.72±0.58 Hz and for ISNs it was 1.8±0.4 Hz (Fig. 3.9A). The group mean firing rates of the WSNs, CSNs and ISNs at corresponding temperatures were correlated. It was observed that the correlation co-efficient of firing rate vs $T_{rec}$ was highly significant for thermosensitive neurons only (Fig. 3.10). The effect of picrotoxin, a GABA-A receptor antagonist, was examined on 58 neurons, of which 37 were thermosensitive (17 WSNs, 20 CSNs) and 21 were ISNs (Table 3.3 & 3.4).
A chi-square test showed that picrotoxin significantly \( (\chi^2 = 14.05, \text{df} \ 4; \ p<0.01) \) affected mPOAH neurons. The mean (+ SEM) firing rates of WSNs, CSNs and ISNs before and after picrotoxin microiontophoretic injection are shown in fig. 3.9) and the percentage change in the firing rate after picrotoxin injection has been shown in (table 3.4). However, more number of the WSNs were inhibited while more number of CSNs were excited by picrotoxin.

Effect of picrotoxin on WSNs:

The effect of microiontophoretic application of picrotoxin was studied on a total of 17 WSNs at a mean (+ S.D.) T_{rec} of 38.02 ± 0.83°C. The scatter plot of the mean firing rates of these WSNs is shown in (Fig. 3.10A). It was observed that 12 neurons \( (p< 0.01) \) were inhibited (Fig. 3.9B), 3 were excited and the rest 2 remained unaffected by picrotoxin (Table 3.3). The group mean (+ SEM.) firing rates of neurons per second during pre- and post-picrotoxin injection period for those which were inhibited were 7.57±1.1 Hz and 1.73 ± 0.6 Hz, for those which were excited were 4.6±0.6 Hz and 6.1 ± 1.4 Hz and for those which did not change were 11.0 ± 3.4 Hz and 11.1 ± 2.9 Hz, respectively. Effect of picrotoxin on a neuronal firing of the WSN is shown in (Fig. 3.11 and Fig. 3.12).

Effect of picrotoxin on CSNs:

The effect of picrotoxin was studied on 20 CSNs at a mean (+ S.D.) T_{rec} of 36.7±1.65°C. The scatter plot of the mean firing rates of these CSNs at respective T_{rec} is shown in (Fig. 3.10B). Out of these neurons, 12 \( (p< 0.01) \) were excited (Fig. 3.9B) and 4 were inhibited by picrotoxin (Table 3.3). The group mean (+ SEM) firing rates of the neurons during pre- and post-picrotoxin injection per second which increased the firing rate by picrotoxin was 1.3±0.29 Hz and 3.77 ± 0.67 Hz, those which were inhibited were 5.71±1.84 Hz and 1.09 ± 0.51 Hz while 3.81 ± 1.0 Hz and 3.78 ± 1.02 Hz for those which remained unaffected. Effect of picrotoxin on a neuronal firing of CSN is shown in (Fig. 3.13 and Fig. 3.14).
Effect of picrotoxin on ISNs:

There were 21 ISNs where the effect of picrotoxin was studied at a mean (± S.D.) $T_{rec}$ of 37.1 ± 1.89°C. The scatter plot of the mean firing rates of these neurons at respective $T_{rec}$ is shown (Fig. 3.10C). Out of these 14 showed increased firing and 6 showed decreased firing rate (Table 3.3). The group mean (± SEM) firing rate per second before and after picrotoxin microinjection was 1.54 ± 0.43 Hz and 2.55 ± 0.35 Hz for those increased while 2.5 ± 0.95 Hz and 1.4 ± 0.74 Hz for those which showed decreased firing. However, the changes in firing rate although were more than 10%, they were not statistically significant.

(b) Microiontophoretic Injection of Prazosin on the mPOAH Neurons:

In this study a total of 118 neurons were recorded from mPOAH. Out of these, 29 (24.5 %) were WSNs, 34 (29 %) were CSNs and 55 (46.5 %) were temperature insensitive neurons (ISNs). The effect of microiontophoretic application of prazosin, an α-1 adrenoceptor blocker, was studied on the spontaneous discharge rate of 65 mPOAH neurons. Out of these neurons, 19 were WSNs and 23 each were CSNs and ISNs. It has been observed that prazosin inhibited both temperature sensitive as well as insensitive neurons. Out of the total recorded neurons, 74 % were inhibited, 14 % were excited and 12 % showed no change by the application of prazosin. Among the thermosensitive group as much as 86 % neurons were inhibited, 7 % were excited and rest 7 % were not affected whereas only 52 % thermo-insensitive neurons were inhibited by prazosin. However, the inhibition of thermosensitive neurons was more significant ($p < 0.001$) than the temperature insensitive neurons ($p < 0.05$). The mean (± SEM) firing rates of WSNs, CSNs and ISNs before and after prazosin microiontophoresic injection are shown in (Fig. 3.17B) and the percentage change in the firing rate after prazosin injection has been shown in (Table 3.6). A chi-square test showed that prazosin significantly ($\Sigma \chi^2 = 13.7$, df 4; $p<0.01$) affected mPOAH neuron7s. The group mean firing rates of the WSNs, CSNs and ISNs at corresponding temperatures were correlated. It was observed that the correlation co-
efficient of firing rate vs $T_{rec}$ was highly significant for thermosensitive neurons only (Fig. 3.18).

**Effect of prazosin on WSNs:**

Out of the 19 WSNs tested, prazosin inhibited significantly the activity of 15 (79\%) neurons. Three neurons were excited (16\%) and one neuron remained unaffected (Table 3.5). Among the 15 inhibited neurons, the firing frequency of 13 neurons decreased by $>50\%$ whereas for the rest 2 neurons, the firing frequency decreased by $<50\%$. The firing frequency of all the 3 neurons which were excited increased by $<50\%$.

**Effect of prazosin on CSNs:**

The effect of prazosin was studied on a total of 23 CSNs. Prazosin significantly inhibited 21 (91\%) neurons and the rest 2 neurons remained unaffected (Table 3.5). The reduction in firing rate of 16 neurons was $>50\%$, while for the rest 5 neurons the reduction was $<50\%$.

**Effect of prazosin on ISNs:**

Out of the 23 ISNs where the effect of prazosin was tested, 12 (52\%) neurons were inhibited, 6 (26\%) were excited and 5 (22\%) remained unaffected (Table 3.5). Among the inhibited neurons all except one was inhibited by $>50\%$. On the other hand, among the excited neurons none except only one neuron was excited by $>50\%$.

[C] **BRAINSTEM STIMULATION STUDY:**

1. **100Hz Stimulation:**

High frequency stimulation (100 Hz) of MRF (100 $\mu$A and 200$\mu$s) induced EEG desynchronization in free moving as well as in anaesthetized condition. Low frequency stimulation (1 Hz) with the current strength up to 600$\mu$A did not induce any visible change in the EEG. High as well as low frequency MRF stimulation affected
the neuronal firing of mPOAH neurons. Such stimulation showed that high frequency stimulation of MRF, influenced both EEG and neuronal firing, whereas, 1 Hz stimulation influenced only the neuronal activity but not the EEG. Effects of high frequency stimulation of MRF were studied on 43 neurons along with the concomitant changes in the EEG. Out of the 43 neurons, 7 were excited, 15 were inhibited and 21 showed no change with MRF stimulation. Moreover, among these 43 neurons, 22 were temperature sensitive and the remaining 21 were temperature insensitive neurons. The results for individual neurons are shown in (Table 3.7). There were six neurons whose firing rates correlated with changes in cortical EEG. The effect of MRF stimulation was also observed on those 6 EEG related neurons (4 related to synchronized EEG and 2 related to desynchronized EEG) (Table 3.9). It was observed that 3 neurons related to synchronized EEG were inhibited (Fig. 3.30) whereas 2 neurons related to desynchronized EEG were excited with 100 Hz stimulation of MRF. The results of individual neurons are shown in (Table 3.9).

2. 1 Hz Stimulation:

The effect of MRF stimulation (1Hz) was studied on the spontaneous discharge rate of 109 mPOAH neurons. Out of these neurons, 25 (23 %) were WSNs, 30 (27.5 %) were CSNs and 54 (49.5 %) were ISNs. The mean firing frequency of WSNs was 6.35 ± 0.73 Hz, CSNs was 4.02 ± 0.71 Hz and of ISNs was 2.9 ± 0.73 Hz at 37°C. The ranges of the latency and duration of the excitatory response induced by MRF stimulation were 5-40 msec and 10-60 msec while for inhibitory responses the latency and duration were 5-25 msec and 25-100 msec, respectively. 1 Hz MRF stimulation generally did not induce any visible change in the EEG.

**Effect of 1 Hz stimulation of MRF on the activity of WSNs:**

Out of the 25 WSNs tested, 22 (88 %) were excited, 2 (8%) were inhibited and 1 (4%) showed no change on MRF stimulation. The latency of excitation was 12.2 ± 1.73 ms (Fig. 3.26).
**Effect of 1 Hz stimulation of MRF on the activity of CSNs:**

The effect of MRF stimulation was studied on a total of 30 CSNs and it was observed that all the 30 CSNs were inhibited by MRF simulation. The duration of inhibition was $63.5 \pm 3.7$ ms (Fig. 3.27).

**Effect of 1 Hz stimulation of MRF on the activity of ISNs:**

Out of the 54 ISNs where the effect of MRF stimulation was tested, 2 neurons were excited, 1 showed inhibition and 51 (22%) remained unaffected. The latency of excitation of 2 ISNs was 5 and 8 ms respectively where as the duration of inhibition of 1 ISN was 55 ms (Fig. 3.28).

**Effect of 100 Hz stimulation of MRF on putative sleep-wake related Neurons:**

Effect of 100 Hz stimulation of MRF was studied on 4 putative sleep related and 2 putative wake related neurons. Out of the 4 putative sleep related neurons 3 were inhibited (Fig. 3.30) and one showed excitation whereas both putative wake related neurons were excited on 100 Hz MRF stimulation. All the inhibited neurons were of cold sensitive type whereas the neurons which were excited comprised majorly of the warm sensitive type. The results of individual neurons are shown in the Table 3.10.

**Effect of 1 Hz stimulation of MRF on putative sleep-wake related Neurons:**

Effect of 1 Hz stimulation of MRF was studied on 6 putative sleep-wake related neurons. Out of those 6 neurons, firing rate of 5 were related to synchronized EEG whereas that of 1 was related to desynchronized EEG. Out of the 5 neurons, 3 were inhibited (Fig. 3.29), one showed excitation and one showed no change with 1 Hz stimulation of MRF. All the inhibited neurons were of cold sensitive type whereas the neuron excited was of warm sensitive type. The results of individual neurons are shown in the Table 3.10.
PUTATIVE SLEEP-WAKE RELATED NEURONS AND PRAZOSIN:

Effect of Prazosin on mPOAH putative sleep related neurons:

There were 3 neurons whose firing rate increased during synchronized EEG. The effect of prazosin was studied on all the three neurons. Out of these, 2 were cold sensitive and one was temperature insensitive neuron. All the three neurons were inhibited by prazosin (Fig. 3.31).

Effect of Prazosin on mPOAH putative wake related neurons:

There were only two neurons related to desynchronized EEG on which the effect of prazosin was studied. One of the neurons was cold sensitive while the other was temperature insensitive. Both the neurons were inhibited by prazosin.

Effect of picrotoxin on mPOAH putative sleep related neurons:

The effect of picrotoxin was studied on three neurons whose firing rate increased during synchronized EEG. Out of these three, two were cold sensitive and one was temperature insensitive. One of the cold sensitive neurons was excited while the other cold sensitive and the temperature insensitive neuron were inhibited by picrotoxin.
SUMMARY OF RESULTS

The results of the study can be summarized as:

MICROINJECTION STUDY:

1. Microinjection of picrotoxin, a GABA-A receptor antagonist, into the mPOAH significantly induced wakefulness and reduced deep sleep as well as REM sleep.
2. Picrotoxin also significantly increased brain and rectal temperature.
3. Pearson Correlation test showed that during hyperthermia a significant linear correlation existed between the brain and rectal temperatures.

MICROIONTOPHORETIC INJECTIONS STUDY:

1. Microiontophoretic application of picrotoxin, a GABA-A receptor blocker, significantly inhibited WSNs, excited CSNs and had no effect on ISNs of the mPOAH.
2. Microiontophoretic application of prazosin, an α-1 adrenoreceptor blocker, significantly inhibited WSNs, CSNs as well as ISNs. However, the inhibition of thermosensitive neurons was much more significant (p< 0.001) than the inhibition of thermo-insensitive neurons (p< 0.05).

STIMULATION STUDY:

1. 1 Hz electrical stimulation of MRF showed that majority of WSNs (88%) were excited, all the CSNs were inhibited whereas majority of temperature insensitive neurons (94%) remained unaffected by MRF stimulation.
2. Out of the total recorded neurons from the mPOAH, it was observed that 30% and 35% neurons were inhibited, 22% and 16% were excited whereas 48% and
49% neurons remained unchanged by 1Hz and 100 Hz stimulation of MRF, respectively.

3. 100 Hz electrical stimulation of MRF induced EEG desynchronization in free moving as well as in the anaesthetized animals. The induced EEG desynchronization outlasted the period of stimulation.

4. 1 Hz electrical stimulation inhibited a majority of putative sleep related neurons.
3.1 This figure shows two halves of photomicrographs of the same rat brain through the mPOAH separated by 500 μm. The tip of the thermocouple (tc) is shown in the left half (A-P - 0.3 mm) while the injection spot (inj.) is shown in the right half (A-P - 0.8 mm) of the sections. Abbreviations of the anatomical terms: ac – anterior commissure, f – fornix, 3v – third ventricle, ox – optic chiasma.
3.2 The anatomical location of all the injection sites are shown in the reconstructed histological sections through medial preoptico-anterior hypothalamic area, as per the atlas of Paxinos and Watson. The filled circles represent the sites of injection of picrotoxin in the mPOAH. Abbreviations: mPOAH: medial preoptico-anterior hypothalamic area, LPO: lateral preoptic area, ac: anterior commissure, f: fornix, ox: optic chiasma, BSTPO: Bed nucleus of stria terminalis preoptic, AH: anterior hypothalamus.
Fig. 3.3 The percentage (mean ± S.E.M.) time spent in wakefulness every hour during baseline, after saline and after picrotoxin microinjection into the mPOAH are represented here. Significance levels as compared to saline, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. 
Fig. 3.4 The mean (± S.E.M.) brain (trace A) and rectal (trace B) temperatures every 30 min during baseline, after saline and after picrotoxin microinjections into the mPOAH are shown this figure. Significance level as compared to saline. * - p < 0.05; ** - p < 0.01 and *** - p < 0.001.
Fig. 3.5 The correlation between the brain and rectal temperatures (mean ± S.E.M.) at every 30 min during baseline, after saline and after picrotoxin microinjections into the mPOAH are shown in this figure. Significance level after picrotoxin microinjection has been compared to that of saline. Significance level of brain temperature, $ -$ significance level of rectal temperature. *, $ - p < 0.05$; **, $$$ - p < 0.01$ and $$$, $$$ - p < 0.001.
Rate of increase and decrease of brain and rectal temperature

![Graph showing the relationship between brain and rectal temperature.]

Pearson Correlation test

![Graph showing the result of the Pearson correlation test.]

Fig. 3.6 [A] The brain temperature has been plotted against the rectal temperature after picrotoxin microinjection into the mPOAH.

[B] Pearson correlation test showing a significant positive correlation between brain and rectal temperatures, after picrotoxin microinjection into the mPOAH is shown here.
Fig. 3.7 This figure shows photomicrograph of a section of rat brain through medial preoptic anterior hypothalamic area showing pontamine sky blue spot (arrow), marked after completion of recording of a warm sensitive neuron. Abbreviations of the anatomical terms: ac – anterior commissure, f – fornix, ox – optic chiasma.
Fig. 3.8 Reconstruction diagram through medial preoptic anterior hypothalamic area, as per the atlas of Paxinos and Watson, is shown in this figure. The stars represent the sites of recording of warm and cold sensitive neurons on which the effect of picrotoxin was seen. Although the neurons were recorded from either side, for convenience, the warm sensitive neurons are shown on the left while the cold sensitive on the right side of the sections. The sites of recording of temperature insensitive neurons have not been shown in this diagram. Abbreviations: mPOAH: medial preoptic-anterior hypothalamic area, lPOA: lateral preoptic area, ac: anterior commissure, f: fornix, ox: optic chiasma, 3v: third ventricle, BSTPO: Bed nucleus of stria terminalis preoptic, AH: anterior hypothalamus.
Fig. 3.9 A. The mean firing frequency (± S.E.M.) of warm sensitive, cold sensitive and temperature insensitive neuronal groups studied are shown here. Horizontal bar within each bar represents the median value of their respective neurons.

B. The mean firing frequency (± S.E.M.) during pre- and post- picrotoxin injection period are shown here. The median value of each of the groups is also shown as horizontal bar in respective columns. Significance levels compared to the pre-injection firing frequency. ** p< 0.01.
**Results**

**Correlation between Temperature and Neuronal Firing**

![Graphs A, B, and C showing correlation between rectal temperature and neuronal firing rates.]

Fig. 3.10 The mean ± S.E.M. value of WSNs, CSNs and ISNs are plotted here. The firing rates were correlated with the change in rectal temperature. The correlation between firing rate and respective temperature of WSNs showed a significant positive correlation and CSNs showed significant negative correlation. The ISNs did not show any significant correlation.
Effect of Picrotoxin on the Neuronal Firing of a Warm Sensitive Neuron

Tbr 37.8 / Trec 36.0 °C

Tbr 38.6 / Trec 37.0 °C

Tbr 39.4 / Trec 38.0 °C

Tbr 39.7 / Trec 39.0 °C

Picrotoxin
(+ve) 80 nA

Current effect
(+ve) 80 nA

Tbr - Brain Temperature
Trec - Rectal Temperature

0 60 120 sec

The neuronal firing of a warm sensitive neuron in mPOAH at different temperatures are shown in this figure which was inhibited by picrotoxin and unaffected by current injection. Tbr and Trec are brain and rectal temperatures, respectively.
Fig. 3.12 The corresponding rate histogram of neuronal firing of same warm sensitive neuron in mPOAH at different temperatures are shown in this figure which was inhibited by picrotoxin and unaffected by current injection. $T_{br}$ and $T_{rec}$ are brain and rectal temperatures, respectively.
**Effect of Picrotoxin on the Neuronal Firing of a Cold Sensitive Neuron**

Fig.3.13 The firing rate of a cold sensitive neuron of mPOAH at different temperatures are shown here. The neuron was excited by iontophoretic application of picrotoxin but was not affected by current injection. $T_{br}$ and $T_{rec}$ are brain and rectal temperature, respectively.
Fig. 3.14 The respective rate histograms of same cold sensitive neuron of mPOAH at different temperatures are shown here. The neuron was excited by iontophoretic application of picrotoxin, however, it was not affected by current injection. $T_b$ and $T_{rc}$ are brain and rectal temperatures, respectively.
Fig. 3.15 This figure shows photomicrograph of a section of rat brain through medial preoptic anterior hypothalamic area showing cannula tract (arrow), the site of recording of a neuron. Abbreviations of the anatomical terms: ac – anterior commissure, f – fornix, ox – optic chiasma, Lv – lateral ventricle, 3v – 3rd ventricle.
Fig. 3.16 Reconstruction diagram through medial preoptic anterior hypothalamic area, as per the atlas of Paxinos and Watson, is shown in this figure. The stars represent the sites of recording of warm and cold sensitive neurons on which the effect of prazosin was seen. Although the neurons were recorded from either side, for convenience, the warm sensitive neurons are shown on the left while the cold sensitive on the right side of the sections. The sites of recording of temperature insensitive neurons have not been shown in this diagram. Abbreviations: mPOAH: medial preoptic-anterior hypothalamic area, lPOA: lateral preoptic area, ac: anterior commissure, f: fornix, ox: optic chiasma, 3v: third ventricle, BSTPO: Bed nucleus of stria terminalis preoptic, AH: anterior hypothalamus.
Mean (± S.E.M.) spontaneous firing frequency of warm, cold and temperature insensitive neurons

**A**

![Bar chart showing mean firing frequency of WSNs, CSNs, and ISNs](chart.png)

<table>
<thead>
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<th>Neuron Type</th>
<th>Pre-Injection</th>
<th>Post-Injection</th>
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<td>WSN</td>
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<td>n = 34</td>
</tr>
<tr>
<td>CSN</td>
<td>n = 34</td>
<td>n = 55</td>
</tr>
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</table>

Mean (± S.E.M.) firing frequency of WSNs, CSNs and ISNs during pre and post prazosin injection

**B**

![Bar chart showing mean firing frequency during pre- and post-prazosin injection](chart2.png)

<table>
<thead>
<tr>
<th>Neuron Type</th>
<th>Pre-Injection</th>
<th>Post-Injection</th>
</tr>
</thead>
<tbody>
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<td>n = 23</td>
</tr>
<tr>
<td>ISN</td>
<td>n = 23</td>
<td>n = 23</td>
</tr>
</tbody>
</table>

Fig. 3.17 [A] The mean firing frequency (± S.E.M.) of total recorded warm sensitive, cold sensitive and temperature insensitive neuronal groups are shown here. Horizontal bar within each bar represents the median value of their respective neurons.

[B] The mean firing frequency (± S.E.M.) during pre- and post-prazosin injection period are shown here. The median value of each of the groups is also shown as horizontal bar in respective columns. Significance levels as compared to the pre-injection firing frequency: * p<0.05, ** p<0.001
**Results**

**Correlation between Temperature and Neuronal Firing**

Fig. 3.18 The mean ± S.E.M. value of WSNs, CSNs and ISNs are plotted here. The firing rates were correlated with the change in rectal temperature. The correlation between firing rate and respective temperature of WSNs showed a significant positive correlation and CSNs showed significant negative correlation. The ISNs did not show any significant correlation.
Results

Effect of Prazosin on Neuronal firing of a Warm Sensitive Neuron

\[ T_{\text{br}} 37.8 / T_{\text{rec}} 36.0 \degree \text{C} \]

\[ T_{\text{br}} 38.6 / T_{\text{rec}} 37.0 \degree \text{C} \]

\[ T_{\text{br}} 39.4 / T_{\text{rec}} 38.0 \degree \text{C} \]

\[ T_{\text{br}} 39.7 / T_{\text{rec}} 39.0 \degree \text{C} \]

\[ T_{\text{br}} 39.7 / T_{\text{rec}} 39.0 \degree \text{C} \]

Prazosin (+ve) 75 nA

Current effect
(+ve) 75 nA

\[ T_{\text{br}} - \text{Brain Temperature} \]
\[ T_{\text{rec}} - \text{Rectal Temperature} \]

Fig. 3.19 The firing rate of a neuron in mPOAH at different brain and rectal temperatures show that it is a representative of a warm sensitive neuron. This warm sensitive neuron was inhibited by prazosin.
Rate Histogram of a Warm Sensitive Neuron

Fig. 3.20 Rate histograms corresponding to the firing frequency of a warm sensitive neuron of mPOAH at different rectal and brain temperatures are shown here. The effect of prazosin, an α-1 adrenoceptor blocker and current effect have also been shown.
Effect of Prazosin on Neuronal Firing of a Cold Sensitive Neuron

\[ T_{br} 38.1 / T_{rec} 38.0 \, ^\circ C \]

\[ T_{br} 37.6 / T_{rec} 37.0 \, ^\circ C \]

\[ T_{br} 36.9 / T_{rec} 36.0 \, ^\circ C \]

\[ T_{br} 36.2 / T_{rec} 35.0 \, ^\circ C \]

\[ T_{br} 36.2 / T_{rec} 35.0 \, ^\circ C \]

\[ \text{Prazosin (+ve) 75 nA} \]

\[ T_{br} 36.2 / T_{rec} 35.0 \, ^\circ C \]

\[ \text{Current effect (+ve) 75 nA} \]

21 The neuronal firing of a cold sensitive neuron in mPOAH at different brain and rectal temperatures are shown here. The effect of prazosin, \( \alpha-1 \) adrenoceptor blocker and current effects show that the neuronal firing rate was inhibited by prazosin.
Fig. 3.22 Rate histograms of respective cold sensitive neuron of mPOAH at different brain and rectal temperatures are shown here. The effect of prazosin, an α-1 adrenoceptor blocker and current effect have also been shown.
Fig. 3.23 This figure shows photomicrograph of a section of rat brain through medial preoptic anterior hypothalamic area showing pontamine sky blue spot (arrow), marked after completion of recording of a neuron. Abbreviations of the anatomical terms: ac – anterior commissure, f – fornix, ox – optic chiasma, inj.- injection site, ct-cannula tract, Lv- lateral ventricle, 3v- 3rd ventricle.
Fig. 3.24 The figure shows the representative histological section of stimulation site in the brainstem. The stimulating electrode track and the lesioned site of stimulation are shown in the photomicrograph of a section through MRF (arrow).
Fig. 3.25 Reconstruction diagram through medial preoptico anterior hypothalamic area, as per the atlas of Paxinos and Watson, is shown in this figure. The stars represent the sites of recording of warm and cold sensitive neurons on which the effect of brainstem stimulation was seen. Although the neurons were recorded from either side, for convenience, the warm sensitive neurons are shown on the left while the cold sensitive on the right side of the sections. The sites of recording of temperature insensitive neurons have not been shown in this diagram. Abbreviations: mPOAH: medial preoptico-anterior hypothalamic area, IPOA: lateral preoptic area, ac: anterior commissure, f: fornix, ox: optic chiasma, 3v: third ventricle, BSTPO: Bed nucleus of stria terminalis preoptic, AII: anterior hypothalamus.
Fig. 3.26 Ten MRI stimulus bound overlapped response of a warm sensitive neuron of mPOAH are shown in this figure. The neuronal firing rate of this neuron was correlated with different rectal and brain temperatures and show that it is a warm sensitive neurons (trace-A). This neuron was excited with the low frequency (1 Hz) MRF stimulation (trace-B). Arrows indicate the stimulus artifact, which represent point of stimulus.
Fig. 3.27 Ten MRI stimulus bound overlapped responses of a cold sensitive neuron of mPOAH are shown in this figure. The neuronal firing rate when correlated with changes in the brain and rectal temperatures it shows that it is a cold sensitive neuron (trace-A) and the neuron was inhibited with 1Hz MRI stimulation (trace-B).
Effect of LF (1 Hz) Stimulation on a Temperature Insensitive Neuron of mPOAH

Fig. 3.28 Ten stimulus-bound response of a temperature insensitive neuron of mPOAH to 1 Hz MRI stimulation are shown in this figure. The neuronal firing rate of this neuron at different rectal and brain temperatures show that it is a representative of temperature insensitive neurons (trace A). This neuron did not show any affect after low frequency (1 Hz) MRF stimulation (trace B).
Effect of LF (1 Hz) Stimulation on a putative Sleep related neuron of mPOAH

![Neuronal Firing](image)

**Fig. 3.29** Ten stimulus bound response of a putative sleep related mPOAH neuron to 1 Hz MRF stimulation are shown in this figure. The neuron showed increased firing during synchronized EEG and decreased firing during de-synchronized EEG (trace-A). This neuron showed inhibitory response by 1 Hz MRF stimulation (trace-B).
Effect of HF (100 Hz) Stimulation on a putative Sleep related neuron of mPOAH

Fig. 3.30 Simultaneous recording of EEG with the spontaneous activity of one putative sleep related neuron from the mPOAH during synchronized and desynchronized EEG are shown in this figure. This neuron was inhibited by high frequency (100 Hz) MRF stimulation (lower trace). This neuron was also inhibited by low frequency (1 Hz) MRF stimulation as shown in earlier figure.
Effect of Prazosin on a Putative Sleep Related Neuron of mPOAH

Fig. 3.1 Simultaneous recording of EEG with the spontaneous activity of one putative sleep related neuron from the mPOAH during synchronized and desynchronized EEG are shown in this figure. The effect of microiontophoretic injection of prazosin, an alpha-1 adrenoceptor blocker and current effect show that the neuronal firing rate was inhibited by prazosin.
### Table 3.1

Mean percentage time (+S.E.M.) spent in SWS, DS and REMS in baseline, saline and picrotoxin injected rats

<table>
<thead>
<tr>
<th></th>
<th>Pre Injection</th>
<th>Post-injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-1 hr.</td>
<td>1 hr.</td>
</tr>
<tr>
<td>Slow-Wave Sleep</td>
<td>16 ± 4.6</td>
<td>31.5 ± 6.6</td>
</tr>
<tr>
<td>Saline</td>
<td>17.0 ± 2.2</td>
<td>25.9 ± 6.9</td>
</tr>
<tr>
<td>Picrotoxin</td>
<td>9.0 ± 3.4</td>
<td>6.7 ± 4.2</td>
</tr>
<tr>
<td>Saline Baseline</td>
<td>5.5 ± 3.5</td>
<td>14.5 ± 3.4</td>
</tr>
<tr>
<td>Deep Sleep</td>
<td>6.6 ± 2.4</td>
<td>6.2 ± 2.2</td>
</tr>
<tr>
<td>Saline</td>
<td>12.2 ± 7.1</td>
<td>1.1 ± 0.7</td>
</tr>
<tr>
<td>Picrotoxin</td>
<td>(***)</td>
<td>(**)</td>
</tr>
<tr>
<td>Saline Baseline</td>
<td>0</td>
<td>0.33 ± 0.2</td>
</tr>
<tr>
<td>REM Sleep</td>
<td>0.2 ± 0.2</td>
<td>1.0 ± 0.6</td>
</tr>
<tr>
<td>Saline</td>
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<td>0.5 ± 0.5</td>
</tr>
<tr>
<td>Picrotoxin</td>
<td>1.9 ± 1.3</td>
<td>0</td>
</tr>
</tbody>
</table>

* Significance is compared to saline. * - p < 0.05, ** - p < 0.01
Table 3.2

Mean percentage time (+ S.E.M.) spent in AW and QW in baseline, saline and picrotoxin injected rats

<table>
<thead>
<tr>
<th>Pre Injection</th>
<th>Post-injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
</tr>
<tr>
<td>1 hr.</td>
<td>48.8±9.5</td>
</tr>
<tr>
<td>2 hr.</td>
<td>39.8±4.7</td>
</tr>
<tr>
<td>3 hr.</td>
<td>43.1±5.1</td>
</tr>
<tr>
<td>4 hr.</td>
<td>34.7±8.3</td>
</tr>
<tr>
<td>5 hr.</td>
<td>33.7±4.8</td>
</tr>
<tr>
<td>6 hr.</td>
<td>26.5±2.5</td>
</tr>
<tr>
<td>7 hr.</td>
<td>29.9±2.4</td>
</tr>
<tr>
<td>8 hr.</td>
<td>31.5±7.3</td>
</tr>
</tbody>
</table>

| Quiet awake   |                |
| Saline        |                |
| 1 hr.         | 38.3±2.4       | 34.3±4.6       |
| 2 hr.         | 29.4±5.3       | 28.4±5.7       |
| 3 hr.         | 42.0±9.2       | 40.9±7.1       |
| 4 hr.         | 47.9±8.0       | 48.5±5.1       |
| 5 hr.         | 38.0±9.4       | 36.3±9.7       |

| Picrotoxin    |                |
| Baseline      |                |
| 1 hr.         | 29.5±5.8       | 12.9±4.4       |
| 2 hr.         | 4.08±1.8       | 3.3±1.4        |
| 3 hr.         | 6.0±2.6        | 6.2±4.1        |
| 4 hr.         | 6.4±2.4        | 7.1±2.9        |
| 5 hr.         | 22.0±6.0       |                |

| Active awake  |                |
| Saline        |                |
| 1 hr.         | 37.6±3.6       | 33.0±6.0       |
| 2 hr.         | 7.6±3.4        | 3.1±1.3        |
| 3 hr.         | 10.3±4.3       | 7.6±3.4        |
| 4 hr.         | 21.3±7.4       | 4.4±1.2        |
| 5 hr.         | 6.8±1.9        |                |

| Picrotoxin    |                |
| Baseline      |                |
| 1 hr.         | 33.6±13.0      | 65.0±9.8       |
| 2 hr.         | 16.1±4.1       | 18.4±6.3       |
| 3 hr.         | 18.8±10        | 14.8±5.5       |
| 4 hr.         | 9.9±5.1        | 10.4±4.3       |

| * Significance as compared to saline. * - p < 0.05, ** - p < 0.01 |
### Table 3.3

**Effects of Picrotoxin on mPOAH neurons**

<table>
<thead>
<tr>
<th>Types of Neuron</th>
<th>Excitation</th>
<th>Inhibition</th>
<th>No Change</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>WSNs</td>
<td>3</td>
<td>12</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>CSNs</td>
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<td>4</td>
<td>20</td>
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<tr>
<td>ISNs</td>
<td>14</td>
<td>6</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>22</td>
<td>7</td>
<td>58</td>
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</table>

### Table 3.4

**Percentage Change in Mean (± S.E.M.) Firing Frequency after Picrotoxin Injection on the mPOAH Temperature Sensitive and Insensitive Neurons.**

<table>
<thead>
<tr>
<th>Types of Neuron</th>
<th>Pre-injection</th>
<th>Post-injection</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>WSNs (n = 12)</td>
<td>7.57 ± 1.1</td>
<td>1.73 ± 0.6**</td>
<td>77.1 %</td>
</tr>
<tr>
<td>CSNs (n = 12)</td>
<td>1.32 ± 0.29</td>
<td>3.77 ± 0.67***</td>
<td>190 %</td>
</tr>
<tr>
<td>ISNs (n = 14)</td>
<td>1.54 ± 0.43</td>
<td>2.8 ± 1.35</td>
<td>81.8 %</td>
</tr>
</tbody>
</table>

Significance level: ** - p < 0.01
### Table 3.5

**Effects of Prazosin on mPOAH neurons**

<table>
<thead>
<tr>
<th>Types of Neuron</th>
<th>Excitation</th>
<th>Inhibition</th>
<th>No Change</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>WSNs</td>
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<td>CSNs</td>
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<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>48</td>
<td>8</td>
<td>65</td>
</tr>
</tbody>
</table>

### Table 3.6

**Percentage Change in Mean (± S.E.M.) Firing Frequency after Prazosin Injection on the mPOAH Temperature Sensitive and Insensitive Neurons**

<table>
<thead>
<tr>
<th>Types of Neuron</th>
<th>Pre-injection</th>
<th>Post-injection</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>WSNs (n = 19)</td>
<td>6.2 ± 0.85</td>
<td>1.48 ± 0.66</td>
<td>76.2 %***</td>
</tr>
<tr>
<td>CSNs (n = 23)</td>
<td>4.2 ± 0.69</td>
<td>1.08 ± 0.29</td>
<td>74.28 %***</td>
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<tr>
<td>ISNs (n = 23)</td>
<td>2.97 ± 0.73</td>
<td>1.28 ± 0.36</td>
<td>56.9 %*</td>
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</table>

Significance level: * - p < 0.05, *** - p < 0.001
Table 3.7

Effect of MRF Stimulation (100 Hz) on mPOAH Thermosensitive and Thermo-insensitive Neurons

<table>
<thead>
<tr>
<th>Types of Neuron</th>
<th>Excitation</th>
<th>Inhibition</th>
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<th>Total</th>
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<tr>
<td>Total</td>
<td>7</td>
<td>15</td>
<td>21</td>
<td>43</td>
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Table 3.8

Effect of MRF Stimulation (1 Hz) on mPOAH Thermosensitive and Thermo-insensitive Neurons

<table>
<thead>
<tr>
<th>Types of Neuron</th>
<th>Excitation</th>
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<th>Total</th>
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<td>Total</td>
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<td>33</td>
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### Table 3.9

Effect of MRF Stimulation (100Hz) on mPOAH Putative Sleep-Wake related Neurons

<table>
<thead>
<tr>
<th>Types of Neuron</th>
<th>Excitation</th>
<th>Inhibition</th>
<th>No Change</th>
<th>Total</th>
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<td>Types of Neuron</td>
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<td>Inhibition</td>
<td>No Change</td>
<td>Total</td>
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<tr>
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</table>
### Table 3.11

**Effect of Prazosin on mPOAH Putative Sleep-Wake related Neurons**

<table>
<thead>
<tr>
<th>Types of Neuron</th>
<th>Excitation</th>
<th>Inhibition</th>
<th>No Change</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>WSNs</td>
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<td>0</td>
</tr>
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### Table 3.12

**Effect of Picrotoxin on mPOAH Sleep-Wake related Neurons**

<table>
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<th>Types of Neuron</th>
<th>Excitation</th>
<th>Inhibition</th>
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<td>CSNs</td>
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<td>1</td>
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</tbody>
</table>
The mPOAH is the only site in the brain where lesion, stimulation and neuronal recording methods all provide evidences that this area is mainly involved in the regulation of sleep and body temperature (McCarty and Szymusiak, 1990; Szymusiak, 1995). A relationship between control of sleep and thermoregulation has also emerged from the studies of brain mechanisms, which advocated the close association between sleep and thermoregulatory mechanisms. GABA and norepinephrine neurotransmitters, in this area have been shown to be mainly involved in the regulation of sleep and body temperature.

Some of the studies suggested the role of GABA in the mPOAH in the regulation of sleep and body temperature but their relative effects on sleep-wakefulness and body temperature has not been studied simultaneously.

It has been reported that GABA in the mPOAH induced hypothermia mainly through GABA-A receptors (Serrano et al., 1985). However, in in vitro system it has been reported that GABA inhibits warm as well as cold sensitive neurons of mPOAH through GABA-A receptors (Yakimova, 1996) but this observation could not explain the possible mechanisms of GABA induced hypothermia as per the existing hypothesis. Therefore, in order to understand the mechanism of action of GABA in thermoregulation at the cellular level, it was all the more important to study the role of GABA individually on warm and cold sensitive neurons in in vivo preparations.

Mallick and Alam (1992) had proposed that sleep is modulated by α-2 adrenoreceptor, wakefulness by β adrenoreceptor, and body temperature by α-1 adrenoreceptor respectively. A part of the proposition that sleep is modulated by α-2 adrenoreceptors has been substantiated by Osaka and Matsumura (1995). Nevertheless, there are some reports, which said that body temperature is also regulated by α-2 adrenoreceptors (Quan et al., 1992). Therefore, it is even more necessary to study the types of adrenoreceptors present on the thermosensitive neurons in the mPOAH.

The hypothalamic warm sensitive neurons have been hypothesized to regulate cycling between Non-REM and REM sleep during sustained sleep.
episodes, via inhibitory modulation of brainstem cell groups (McGinty and Szymusiak, 1990). It has been shown that the brainstem area, which affects sleep-wakefulness also influences neurons of the mPOAH (Mallick et al., 1986; Boulant and Demeiville, 1977). Hence, it is likely that thermosensitive neurons in the mPOAH may also be modulated by the brainstem along with simultaneous changes in the EEG.

**Proportion of Thermosensitive Neurons**

It has been observed in this study that in *in vivo* preparation and under urethane anaesthesia there are about 53% thermosensitive neurons in the mPOAH of rats. This is comparable to 52% and 65.5% thermosensitive neurons reported in rats (Hori et al., 1988) and rabbits (Boulant and Demieville, 1977), respectively. Thermosensitive neurons have been recorded in several species at various locations and in different types of preparations, including anaesthetized and unanaesthetized animals, in *in vitro* tissue slices and in tissue culture. The proportion and location of thermosensitive neurons recorded in many of the electrophysiological studies have been shown in detail in Table 1 in the review of literature sections.

In slice preparation a wide variation in firing rates have been reported by different groups (Boulant and Dean, 1986). In this study, among the recorded neurons, about 25% were WSNs while about 29% were CSNs. The differences in percentages of warm and cold sensitive neurons in this study compared to other reported studies could be attributed to a number of factors viz. the species studied, type and depth of anaesthesia at the time of recording, *in vivo* or *in vitro* (slice) preparation and extent of the anatomical area explored for recording.

Tissue slice studies have mapped the locations of thermosensitive neurons in the mammalian hypothalamus. Frontal preoptic slices showed that warm-sensitive neurons were located primarily in the central and lateral portions of medial and lateral preoptic areas and that cold sensitive neurons were present mainly in the central portion of the medial preoptic area (Silva and Boulant, 1984;
1986). The extent of area may affect population of neurons of varying sensitivity because of the presence of heterogeneous population of neurons and their afferent and efferent connections. This may be supported by the reports that the intensity of effects of medial and the lateral preoptic areas varied significantly on sleep-wakefulness and on temperature regulation in rats (Alam and Mallick, 1990, 1991, 1994; Mallick and Alam, 1992).

**Effect of Picrotoxin, a GABA-A receptor antagonist**

It was observed in this study that picrotoxin microinjection into the mPOAH increased wakefulness as well as body temperature. Since both the functions are known to be modulated by the mPOAH, it is reasonable that they were significantly affected. However, the effects were not nonspecific due to physical interference or permanent damage to the mPOAH because the effects were reversible which lasted for about 5-6 hours post-injection and then returned to baseline. On the other hand, the effects were specific to picrotoxin because, saline injection into the same site did not significantly affect either of those parameters. It may be clarified that saline affected S-W and $T_{rec}$ non-specifically for approximately half an hour as has been reported in a series of studies from this laboratory (Alam and Mallick, 1990, 1991; Mallick and Alam, 1992; Mallick and Joseph, 1997, 1998). However, in this study when compared at 1 hour, the effects were possibly neutralised and the difference did not reach the level of statistical significance. Nevertheless, in some studies (Mendelson et al., 1992) the non-specific effect after microinjection in the preoptic area lasted for a longer duration. Since these were non-specific effects (as the other authors also suggested) it is difficult to attribute any specific reason for it. Picrotoxin, a GABA-A blocker, significantly affected both sleep-wakefulness and body temperature. Hence, it is likely that GABA in the mPOAH is normally acting through the GABA-A receptors for spontaneous regulation of those functions. It may be argued that since picrotoxin is a chloride channel blocker, it could also block any other chloride channel e. g. those sensitive to glycine (Karlsson et al., 1997).
However, it has been reported that GABA but not glycine in the preoptic area affected thermoregulation (Yakimova, 1989). Therefore, it is likely that in this study picrotoxin blocked GABA mediated chloride channel. Although microinjection with remote control pump has been established, manual injection was preferred in this study so that specific and non-specific results from previous independent studies where different agonists and antagonists of several neurotransmitters including that of GABA were manually injected could be compared.

The effects on either sleep-wakefulness or body temperature may be supported by earlier independent isolated reports where it has been shown that GABA-ergic mechanism in the mPOAH may affect sleep-wakefulness (Mendelson et al., 1989; Ali et al., 1999) and body temperature (Osborne et al., 1994). Intraperitoneal administration of muscimol, a GABA-A receptor agonist, in the rats induced a decrease in wakefulness and an increase in sleep (Lancel, 1999). A comparable finding was also observed in humans where a single oral dose of Gaboxadol (THIP), a partial GABA-A receptor agonist, was found to significantly increase sleep (Faulhaber et al., 1997). Based on the results of this study it may be said that the effect of GABA in those studies might have been mediated through mPOAH. Microinjection of picrotoxin into the mPOAH is effectively similar to that of a reduction in the level of GABA. This view may be supported by the fact that the GABA level was found to decrease in the microdialysed samples collected from the septal region of basal forebrain during wakefulness in freely moving normally behaving cats (Mallick et al., 1997). Thus, it may be said that GABA in the mPOAH is normally active to induce sleep and to reduce excessive activity.

Picrotoxin not only increased wakefulness but also induced hyperthermia. Central and systemic application of GABA and GABA-agonists caused a fall in core temperature, while antagonists induced hyperthermia (Serrano et al., 1985; Clark and Lipton, 1985). Therefore, it may be said that the effects of systemic application of GABA agonist and antagonist might have been mediated through the mPOAH. It has been reported that muscimol, a GABA-A agonist, decreased the tonic activity of a majority of warm sensitive neurons in the mPOAH in contrast to bicuculline, a GABA-A antagonist (Yakimova et al., 1996). In another recent study it has been
shown that synaptic transmission through Ca\(^{2+}\)-independent release of neurotransmitter on the warm sensitive neurons was completely blocked by GABA-A receptor antagonist which in turn might modulate directly or indirectly tonic firing of the warm sensitive neurons (Hori et al., 1999). Based on these reports the results of this study may be interpreted as that picrotoxin might have blocked the activity of the warm sensitive neurons in the mPOAH resulting in hyperthermia. Therefore, it needed to be confirmed if the thermosensitive neurons in the mPOAH possessed GABA-A receptors. Results of microiontophoretic injection of picrotoxin on the thermosensitive neurons support such possibility (discussed later).

Both the brain and the rectal temperatures were increased after picrotoxin injection into the mPOAH. The correlation analysis showed that the rate of increase of brain and rectal temperatures went hand in hand for 2 hours. Thereafter, the rate of increase of rectal temperature was higher than that of the brain temperature. The Pearson correlation test indicated that both brain and rectal temperatures had a tendency to increase simultaneously, however, the brain temperature varied within a relatively narrower range as compared to that of the rectal temperature. This suggests that the brain has a better heat dissipating mechanism and rightly so considering its sensitive nature. It may also be argued that physiologically the brain may not be capable of withstanding a wider variation in temperature, however, it has the ability to redistribute the heat to peripheral parts of the body. A rapid decrease in brain tissue pH at higher temperature (Katsumura et al., 1995) may also be an underlying possible mechanism for a better heat dissipating ability of the brain.

After picrotoxin microinjection into the mPOAH the wakefulness and the hyperthermia went hand in hand which lasted for 5 and 6 hours, respectively. The duration of effect was comparable to the previous results of picrotoxin microinjection in different regions of the brain under similar conditions (Kaur et al., 1997; Ali et al., 1999). The wakefulness lasted longer than that of active movement suggesting that neither wakefulness nor hyperthermia were due to increased movement or related muscular activity. The changes in wakefulness and increased body temperature were temporally correlated. The mPOAH might have either influenced both the functions simultaneously or the primary effect was on one of the functions, which in turn
affected the other function. Both these phenomena may be supported by previous reports. It has been reported that although norepinephrine in the mPOAH might independently modulate sleep-wakefulness and body temperature (Mallick and Alam, 1992), acetylcholine might not (Mallick and Joseph, 1997). Also, the body temperature rises during wakefulness and decreases during sleep (McGinty and Szymusiak, 1990). While the increased body temperature (or warming) induced sleep (Nakao et al., 1995; Roberts and Robinson, 1969), a decreased body temperature (or cooling) induced wakefulness (Szymusiak and Satinoff, 1984). Therefore, although the results of this study showed that picrotoxin simultaneously modulated sleep-wakefulness and body temperature, it could not be established if there was any cause and effect relationship between these changes.

The injection of picrotoxin induced wakefulness and hyperthermia for a comparable length of time. This suggests either or both the following two possibilities. First, that GABA-A receptors are present on both sleep-wake related neurons as well as on temperature sensitive neurons and second, that GABA-A receptors are present on neurons related to either sleep-wakefulness or thermoregulation so as to affect either of the functions. However, since, sleep-wakefulness and thermoregulation are known to influence each other, both the functions were affected. There are evidences to show that in the mPOAH some of the sleep related neurons are GABA-ergic (Gallopin et al., 2000; Szymusiak, 1995) and in vitro (Yakimova et al., 1996) as well as results of our in vivo studies have shown that temperature sensitive neurons are GABA-ceptive. The mPOAH may receive GABA-ergic inputs from distant sites (Szymusiak, 1995) or from local inter-neurons (Tappaz, 1977; Brownstein, 1977). Since picrotoxin is an antagonist of GABA-A receptor and GABA is an inhibitory neurotransmitter, it may be interpreted that normally GABA is active in the mPOAH to keep those functions inhibited or at a low level so that when the GABA-A receptors were blocked, opposite responses were expressed. It may also be said that normally GABA in the mPOAH prevents the subjects to go to excessive wakefulness (or excitable state) and associated hyperthermic condition or vice-versa.
Effect of Picrotoxin on mPOAH Thermosensitive Neurons

The effects of picrotoxin, a GABA-A receptor blocker, has been studied on the thermosensitive and insensitive neurons of the mPOAH. Out of the total number of mPOAH neurons tested about half (50%) increased while 38% decreased their firing rate by more than 10% of their respective firing rate and the rest 12% neurons remained unaffected by microiontophoretic application of picrotoxin, a GABA-A receptor antagonist. Among the ISNs although 95% changed their firing rate by more than 10%, the group mean firing rates of the neurons that increased and decreased firing rates were statistically insignificant. In contrast, picrotoxin significantly affected the spontaneous firing rate of a majority of the WSNs as well as the CSNs, however, about 15% remained unaffected. These observations suggest that one, the results were not non-specific and two, that GABA is only one of the components which, in association with others, regulates body temperature. Although picrotoxin had excitatory as well as inhibitory effects on both the WSNs as well as the CSNs, a majority of the WSNs (71%) were inhibited while a majority of the CSNs (60%) were excited. This result is in contrast to that of the microiontophoretic application of prazosin on thermosensitive neurons, which under similar conditions had inhibitory effects on both the types of thermosensitive neurons. Thus, it may possibly be said that in general GABA exerts opposite influence on the thermosensitive neurons; the WSNs were excited while the CSNs were inhibited.

This study was done in in vivo condition where, unlike that of the slice preparation studies, the responsiveness of the neurons to peripheral inputs, thermal or otherwise, were intact. Besides, in this study specific response of a neuron to iontophoretic application of picrotoxin in the immediate vicinity of the recorded neuron (without influencing other neurons situated at a distance) could be studied unlike in slice preparation where normally the concentration of the drugs are altered in the bath in which the slices are kept. In such preparation, neurons at a distance, than that being recorded, are also affected by the test chemicals. Thus, in
In the latter case, the influence of the drug on other neurons in the slice and its influence on the activity of the neurons being studied could not be ruled out. The effects were specific to picrotoxin injection since an equivalent quantity of current injection through the 3M KCl barrel was ineffective. 3M KCl was taken in the control barrel because the recording barrel had sodium salt and it has been reported that hypothalamic cells were sensitive to Na⁺ (Stone; 1985). However, it may be argued that the pH and osmolarity of the KCl solution were different than that of picrotoxin solution.

Within every group, picrotoxin inhibited the neurons whose mean firing rate was relatively higher than those having lower firing rates. Nevertheless, it does not support that the firing rate of the neurons was the deciding factor for picrotoxin to induce the response. The mean firing rate of WSNs which were excited was comparable to that of the mean firing rate of the CSNs which were inhibited. Since picrotoxin effect was studied on the neurons within comparable temperature range at random (the range of temperature is mentioned in the results), it is unlikely to be dependent on the temperature at which picrotoxin was applied. This may also be supported by the fact that there were four neurons (1WSN, 2CSNs and 1ISN) where picrotoxin injection was repeated at two different temperatures when the pre-injection firing rates were different. It was observed that at both temperatures WSNs decreased, CSNs increased and ISNs remained unaffected. Picrotoxin affected the spontaneous firing rate of temperature sensitive as well as insensitive neurons in the mPOAH. It suggests that normally GABA is spontaneously active in the mPOAH for the regulation of several physiological functions. It has been reported that central or systemic administration of GABA and its agonists caused a fall in core temperature (Bligh, 1981), while antagonists induced hyperthermia (Clark and Lipton, 1985; Serrano et al., 1985). The results may be supported by the fact that microinjection of GABA agonist or its antagonist into the POAH affected sleep (Ali et al., 1999) and body temperature (Bligh, 1981). Presence of GABA has been demonstrated in relatively high concentration in various hypothalamic nuclei and it appears to be mainly associated with intrinsic hypothalamic neurons (Ottersen and Storm-Mathisen, 1984). Also that GABA-A receptors are present on
the hypothalamic neurons (Fenelon and Herbison, 1996) and hypothalamic neurons are sensitive to picrotoxin (Barone et al., 1994) support the present findings. GABA level has been reported to alter in the basal forebrain including the POAH in relation to several functions including changes in body temperature (Ohtani et al., 1999) and sleep-wakefulness (Mallick et al., 1997).

Excitation and inhibition of the CSNs and WSNs respectively, by picrotoxin suggest that normally GABA has an inhibitory and excitatory effect, respectively, on CSNs and WSNs. GABA is known to be an inhibitory neurotransmitter and it inhibits the spontaneous firing rate of thermosensitive neurons in the POAH through GABA-A receptors (Yakimova et al., 1996). Hence, it is reasonable that picrotoxin induced an excitatory effect on CSNs, however, inhibition of WSNs by picrotoxin apparently suggests that normally GABA is excitatory to those neurons.

There could be two possibilities:

i) GABA excites WSNs or

ii) GABA modulates the spontaneous release of an other neurotransmitter at the pre-synaptic terminal having an excitatory effect on the recorded WSNs.

The first possibility may be excluded because in in vitro preparation GABA has been reported to inhibit WSNs in POAH through its action on GABA-A receptor (Yakimova et al., 1996). It is being proposed that the second possibility may be true for the action of GABA in the mPOAH, which may be explained as follows.

The mPOAH neurons receive excitatory norepinephrinergic inputs (Tanaka et al., 1992; Saphier, 1993). It has been reported in the hippocampal synaptosome preparation that GABA evoked norepinephrine release by activating GABA-A receptors and GABA transporters (Pascio et al., 1999). Therefore, it is being proposed that in normal situation, in the mPOAH, GABA acts on pre-synaptic GABA-A heteroreceptors and causes release of norepinephrine, which excites the
WSNs. Thus, in this study, when GABA-A receptors were blocked by picrotoxin the norepinephrine release was reduced which resulted in inhibition of the WSNs. Since picrotoxin was injected iontophoretically from a barrel in close apposition to that of the recording barrel, norepinephrine release must be modulated by picrotoxin acting on GABA-receptor present very close to the recording neuron. This is possible if there was norepinephrine-ergic terminal on the recording neuron and the norepinephrine terminal possessed GABA-heteroreceptor. This model may be supported by the fact that adrenergic terminals have been reported in close apposition of POAH neurons (Reuss et al., 1999) and neuron terminals in POAH possess GABA-A receptors (Fenelon and Herbison, 1996). However, it needs to be experimentally confirmed that WSNs in the mPOAH receive adrenergic terminals that also possess GABA-ergic heteroreceptors. It is also important to mention that picrotoxin blocks chloride channels sensitive to glycine as well as GABA (Karlsson et al., 1997). Hence, to confirm our hypothesis for presence of GABA-A heteroreceptors, one needs to test with specific GABA-A blockers. Nevertheless, presence of GABA-A receptors in our model may reasonably be supported by the facts that GABA but not glycine in preoptic area affected body temperature (Yakimova and Ovtcharov, 1989) and in this study the neurons sensitive to temperature were significantly affected by picrotoxin.

The results of this study suggest that GABA in the mPOAH may have a direct inhibitory action on the CSNs while it has an indirect norepinephrine mediated excitatory action on the WSNs for the regulation of body temperature. The proposition may be supported by the fact that the concentration of both norepinephrine and GABA decreased after exposure of rats to cold (Ohtani et al., 1999) and also that norepinephrine concentration increased during heat stress (Myers and Chinn, 1973). On the other hand, it has been reported that GABA and norepinephrine both evoked hypothermia (Poole and Stephenson, 1979; Clark and Lipton, 1985, Serrano et al., 1985). Thus, when there is increased concentration of GABA it activates the CSNs and deactivates the WSNs (through norepinephrine) causing stimulation of hypothermic responses so that the temperature tends to increase while during reduced release of GABA opposite responses are triggered.
for the regulation of body temperature. Therefore, it is reasonable to hypothesize that GABA in mPOAH activates directly CSNs and indirectly WSNs and switches on or switches off heat dissipating and heat generating mechanisms, respectively, for thermoregulation.

**Effect of Prazosin on mPOAH Thermosensitive Neurons**

Microiontophoretic application of prazosin, an α-1 adrenoceptors blocker was tested on the mPOAH thermosensitive and insensitive neurons, both. Prazosin affected spontaneous firing rate of 93% of the thermosensitive neurons in the mPOAH suggesting that the thermosensitive neurons possess α-1 adrenoceptors. This confirms the hypothesis based on microinjection studies proposed earlier from our laboratory that NE in the mPOAH regulates body temperature by acting on α-1 adrenoceptors (Mallick and Alam, 1992). It must be mentioned that 78% of the temperature insensitive neurons were also affected by prazosin. This is because non-thermoregulatory function of the mPOAH may also be modulated by NE (Daftary et al., 1998, 2000). It is important to note that among the affected neurons, a majority of the inhibited neurons were thermosensitive type while a majority of the excited neurons were temperature insensitive type. All the affected CSNs were inhibited. Among the affected WSNs, although a majority was inhibited, a small number was excited by prazosin.

The firing rates of the WSNs and CSNs are reciprocally related, directly or indirectly, so that at higher temperature the former increased while the latter decreased their firing rates and they behaved in an opposite manner at lower temperature (Hori et al., 1991). Since thermosensitive neurons were inhibited by prazosin, it is likely that the action of NE on these neurons are excitatory and also that in normal situation NE is spontaneously and tonically active in mPOAH for body temperature regulation at least in rats. It has been shown that noradrenergic terminal (Reuss et al., 1999) and adrenoceptors (Palacios et al., 1987) are present on the POAH neurons. In *in vitro* slice preparation NE has been shown to be excitatory to
the thermosensitive neurons (Sun et al., 1997). These reports support the present results. However, in those studies the subtype of adrenoceptor involved in thermoregulation was not investigated. It has also been reported that the POAH neurons receive NE-ergic inputs from locus coeruleus (Tanaka et al., 1992; Saphier, 1993; Bia and Renaud, 1998) and effects of stimulation of locus coeruleus on the POAH neurons could be blocked by adrenoceptor antagonist (Osaka and Matsumura, 1994). Hence, it may be said that these inputs are possibly involved in POAH mediated adrenergic regulation of the body temperature and we propose that the effects are mediated through α-1 adrenoceptors.

Isolated studies have reported that NE or its agonist induced hypothermia (Mallick and Alam, 1992; Poole and Stephenson, 1979) while its antagonist induced hyperthermia (Mallick and Alam, 1992). On the other hand, NE concentration increased during heat stress (Myres and Chinn, 1973) and it reduced during lowered environmental temperature (Ohtani et al., 1999). Therefore, considering the results of this study together, with previous reports it may be said that for different concentrations of NE to activate different types of thermosensitive neurons (WSNs and CSNs), it is likely that the sensitivity of the thermosensitive neurons to NE would change during different thermal conditions. One of the possibilities could be that the WSNs are responsive to higher concentration while that the CSNs to lower concentration of NE. This view may be supported by the fact that in cold stressed rats, POAH neurons become more sensitive to NE (Sun et al., 1997) i.e. CSNs are likely to be more sensitive to lesser concentration of NE. Also, it has been reported in in vitro experiments that warming reduced the duration of pre-potential of the WSNs resulting in increased firing rate of these neurons (Griffin et al., 1996) indirectly, suggesting a change in sensitivity of thermosensitive neurons. Hence, it is being proposed that there is an optimum level of NE in the mPOAH and both the WSNs and CSNs are moderately active to maintain the set point for thermoregulation. When the temperature changes, depending on high or low, there will be an increase or decrease in NE concentration in the system, respectively and the sensitivity of the neurons would change. This altered level of NE would then activate the WSNs or CSNs as the case may be, leading to stimulation of hypo- or hyper-thermic responses,
respectively. This will tend to bring back the temperature to normal level resulting in maintenance of the body temperature.

It has been reported that adrenoceptor numbers are altered in relation to changes in NE concentration (Dausse et al., 1982). Hence, alteration of thermosensitivity may be modulated by the number of adrenoceptors present on the neurons, which in turn depends on the concentration of NE. An increase in NE (Myers and Chinn, 1973) and panting (Hori et al., 1991) during higher temperature and increased panting by NE (Morilak et al., 1987) fit in with the above mentioned proposition. Functionally it may be said that since the NE-ergic REM-OFF neurons cease firing during REM sleep, there will be a reduction of NE in the projected area, including the POAH where the thermosensitive neurons are located. This reduced concentration of NE would stimulate the CSNs (since body temperature falls during deep sleep and CSNs are responsive to lower concentration of NE) which in turn would activate the hyperthermic response causing an increase in body temperature during REM sleep (Parmeggiani, 1980). This response is possibly reflected as a fluctuation in body temperature during REM sleep (Alam et al., 1995). Another possibility is that one of the thermosensitive neurons is directly affected by NE and the other type is affected indirectly through the mediation of closely placed interneuron, may be GABA, detail of which needs to be investigated further.

**Effect of Brainstem Stimulation on Thermosensitive Neurons**

The effects of high (100 Hz) as well as low (1 Hz) frequency electrical stimulation of Ascending Reticular Activating System (ARAS) on the thermosensitive and insensitive neurons of the mPOAH were studied. The effect of high frequency electrical stimulation of brainstem on cortical activation was also observed in both free moving and in anaesthetised animals. Following high frequency stimulation the effects on the neuronal firing along with simultaneous change in cortical EEG was also observed in anaesthetised condition. It was observed in this study that 50% of the neurons in the mPOAH were thermosensitive and among them 45% were WSNs and 55% were CSNs. Out of the total number of recorded neurons
from the mPOAH, 30% and 35% were inhibited, 22% and 16% were excited whereas 48% and 49% neurons remained unaffected by 1Hz and 100 Hz stimulation, respectively. The excitatory or inhibitory effects on the POAH neurons have been observed following low and high frequency electrical stimulation of brainstem (Fenske et al., 1975; Gardner and Phillips, 1977; Kawakami et al., 1979; Mallick et al., 1984; Kumar et al., 1984). However, in none of the earlier studies, the specificity of the neuronal behaviour to changes in physiological responses was correlated.

In this study, the effect of low frequency (1Hz) brainstem stimulation on thermosensitive and insensitive mPOAH neurons showed that a majority of WSNs (88%) were excited, all the CSNs were inhibited whereas a majority of temperature insensitive neurons (94%) remained unaffected. Previously it was shown that 50% WSNs and 57% CSNs in the POAH and septum received excitatory inputs from the brainstem (Boulant and Demieville, 1977). As observed in this study, the primary excitation of WSNs of the mPOAH in response to single pulse brainstem stimulation agrees well with the observation of Boulant and Demieville (1977) in rabbits, but the inhibition of CSNs in response to brainstem stimulation was opposite to their findings. The discrepancy in the observed effects in this study could be attributed to the species difference or due to the fact that the neurons of the septal and medial preoptic regions were not analysed separately by them. Another possibility may be that either the area of stimulation of the brainstem was not identical or that the studies were done at different temperatures. Boulant and Demieville (1977) had suggested that CSNs in the POAH received a greater proportion of inputs from the brainstem than the WSNs and this finding is comparable to the results of this study as it has been observed that all the CSNs were inhibited by brainstem stimulation. Our other study that GABA by acting through GABA-A receptors modulate the firing rates of thermosensitive neurons in opposite manner i.e. excitation and inhibition of WSNs and CSNs, respectively (Jha et al., 2001) and the brainstem stimulation induced responses on the thermosensitive neurons were similar.

High frequency MRF stimulation induced EEG desynchronization and wakefulness as was observed in previous studies (Moruzzi, 1972; Mallick et al., 1986; Thankachan et al., 1999) this suggested that the stimulating electrode was
located in the Ascending Reticular Activating System (ARAS) (Moruzzi, 1972). It was observed that a) high frequency brainstem stimulation affected more than 50% thermosensitive neurons, whereas a majority of thermo-insensitive neurons remained unaffected. A similar number of neurons were also affected by 1 Hz stimulation. b) changes in the firing rates of thermosensitive neurons could be observed along with concomitant changes in the EEG. It was reported that mPOAH neurons were inhibited by high frequency (100Hz) brainstem stimulation along with simultaneous change in cortical EEG (Kumar et al., 1984). Low frequency brainstem stimulation generally did not induce any visible changes in EEG and it could also be observed in this study. Both excitatory and inhibitory responses of POAH neurons to low frequency stimulation of brainstem were reported by Mallick et al., (1984). It was observed that electrical stimulation of locus coeruleus, a part of the brainstem, inhibited and excited sleep and wake related neurons in the POAH, respectively (Osaka and Matsumura, 1994). It has also been shown that orthodromic excitatory responses were evoked in the majority of basal forebrain neurons following brainstem stimulation (Alam et al., 1995). Conversely, electrical stimulation of mPOAH elicited inhibitory effects on the wake-related neurons in the wake inducing areas in the brainstem (De Armond and Fusco, 1971; Szymusiak and McGinty, 1989). These reports suggested reciprocal projections between brainstem and mPOAH. However, results of these earlier studies fail to show if the wake inducing area has any direct or indirect effect on pre-identified thermosensitive neurons in the mPOAH. Our results showed that brainstem exerts differential influence on the thermosensitive neurons i.e., WSNs were excited while CSNs were inhibited along with cortical activation.

Thermal stimulation studies have also suggested that peripheral thermal signals converge on the reticulospinal neurons in the midbrain (Hori and Harada, 1976). It was observed that mesencephalic reticular neurons were involved in the thermoregulatory muscle tone and shivering. Both these responses have been reported to increase after glutamate microinjection into this area (Toyoko, et al., 1988). Furthermore, it has also been observed that pontine tegmental lesioned animals became more susceptible to thermal loads i.e., the thresholds for shivering (heat-gain) and panting (heat-loss) were lowered in those animals (Amini-Sereshki and Morrison,
1986). It has also been suggested that thermoregulation in those pontine lesioned animals was lowered during sleep. Brainstem noradrenergic locus coeruleus (LC) neurons have been reported to participate in physiological responses to thermoregulatory challenges e.g. panting (Morilak, 1987). These studies indicated that brainstem neurons influenced the thermoregulatory responses.

There are reports indicating that the basal forebrain and frontal cortex receive profuse projections from large numbers of reticular and locus coeruleus cells (Jones and Yang, 1985). Both cholinergic and noradrenergic neurons of reticular activating system send projections to a large number of areas. Cholinergic projections ascend to the substantia nigra and other basal ganglia structures, to the basal forebrain including hypothalamus, thalamus and cerebellum (Garcia-Rill, 1991; Reese et al., 1995). The noradrenergic projections have parallel inputs to all these sites, but in addition, send ascending projections directly to the cerebral cortex (Aston-Jones et al., 1991).

Anatomical and physiological evidences showed that mPOAH sleep related cell types along with thermosensitive neurons promote sleep, in part, via descending inhibitory actions on arousal system, localized in the brainstem, posterior hypothalamus and magnocellular basal forebrain (Szymusiak, 1995). This study indicates that the reticular activating system plays a critical role in the modulation of activity of thermosensitive neurons of the mPOAH. Thermal stimulation of mPOAH elicited appropriate thermoregulatory responses such as sweating, panting, etc (Boulant and Dean, 1986; Hori, 1991). At the same time focal warming of mPOAH inhibited wake related neurons in the MRF and posterior hypothalamus (De Armond and Fusco, 1971; Krilowicz et al., 1994) and induced sleep (Roberts and Robinson, 1962). Conversely, following brainstem stimulation, inhibition of sleep related neurons in the mPOAH have been observed. Reciprocal interactions between the brainstem and mPOAH possibly have strong influence on thermoregulatory control of sleep. A subpopulation of mPOAH warm-sensitive neurons displayed sleep related while cold-sensitive neurons displayed wake related discharge patterns in cats and rats (Alam et al., 1995; 1997). Therefore, it is possible that during sleep, increased firing of mPOAH WSNs inhibited the wake related brainstem neurons thereby maintaining sleep. On the other hand, brainstem stimulation induced inhibition of
sleep related neurons in the mPOAH indicated that possibly wake related neurons in the brainstem inhibited sleep related neurons in the mPOAH to induce wakefulness via ascending projections. In this study, apart from the effects on the thermosensitive neurons, brainstem stimulation induced response could also be observed on some of the putative sleep related thermosensitive neurons. The observation of the inhibition of these putative neurons by brainstem stimulation was supported by the above proposition. It has been reported that some of the sleep related neurons are also thermosensitive (Alam et al, 1995) and brainstem area is partially involved in thermoregulation. Therefore, it is reasonable that brainstem neurons may modulate the thermosensitive neurons as well.

Possible mechanisms of NE, GABA and brainstem mediated influences on the thermosensitive neurons in the mPOAH

NE mediated responses on thermosensitive neurons:

Microiontophoretic application of prazosin, an α-1 adrenoreceptors blocker, on the mPOAH WSNs and CSNs had inhibitory effects on both the types of thermosensitive neurons. Hence, NE excites both types of thermosensitive neurons in the mPOAH. Independent isolated studies have shown that NE induced hypothermia. The WSNs have been implicated in heat dissipating mechanisms whereas CSNs in heat generating mechanisms. However, our results indicated that NE excited both types of thermosensitive neurons. The mechanism of action of NE on thermosensitive neurons may be as followed. As explained earlier in the discussion that NE concentration changes in the mPOAH during hypo- and hyperthermic conditions and the sensitivity of WSNs and CSNs is modulated by changes in concentration of NE. However, the altered concentration of NE in mPOAH at different body temperature excites the WSNs or the CSNs, respectively and maintains the body temperature at a normal level.
**GABA mediated responses on thermosensitive neurons:**

Microiontophoretic application of picrotoxin, a GABA-A receptor antagonist, on the thermosensitive neurons had inhibitory and excitatory responses on WSNs and CSNs, respectively. Thus, it may possibly be said that in general GABA exerts different influence on the thermosensitive neurons; the WSNs were excited while the CSNs were inhibited. It has been observed that GABA microinjection into the mPOAH induced hypothermia. Therefore, the excitation and inhibition of WSNs and CSNs respectively, may be the likely mechanism of GABA induced hypothermia. However, since, GABA is known to be an inhibitory neurotransmitter (Decavel and Van den Pol 1990), it might not be directly inhibiting the WSNs. The possible mechanism of GABA induced excitation of WSNs may only be explained along with the interaction of other neurotransmitter(s).

**Brainstem stimulation induced responses on thermosensitive neurons:**

The WSNs and CSNs were excited and inhibited, respectively, following brainstem stimulation. The results of this study have provided the electrophysiological evidence for the types of projections between brainstem and mPOAH. The results have further helped to propose a model for connection between neurons.

**Integration of NE and GABA effects on thermosensitive neurons in the mPOAH**

The results of this study showed that NE by acting on α-1 adrenoreceptors excited both WSNs and CSNs. It is known that activation of WSNs induces hypothermia whereas activation of CSNs induces hyperthermia (Hori et al., 1991). Isolated studies have reported that NE and its agonist induced hypothermia while its
Antagonist induced hyperthermia (Poole and Stephenson, 1979; Mallick and Alam, 1992). Although NE mediated excitation of WSNs, agrees well with the earlier report that NE induced hypothermia, NE mediated excitation of the CSNs does not fit in. On the other hand, it has been observed in this study that GABA in the mPOAH induced hypothermia and at the cellular level it exerted opposite effects on WSNs and CSNs. The effects of GABA agrees well with the concept that excitation and inhibition of WSNs and CSNs could be the likely mechanism of GABA induced hypothermia. However, since GABA is known to be an inhibitory neurotransmitter (Decavel and Van den Pol, 1990) it is unlikely that GABA would excite WSNs directly. Hence, the cellular mechanism of action of GABA and NE individually on thermosensitive neurons is not as simple. However, since GABAergic neurons are often inter-neurons, it has been attempted to explain the modulation of thermosensitive neuronal responses combining the effects of the two neurotransmitters.

It has been reported that (a) the concentration of both norepinephrine and GABA decreased after exposure of rats to cold (Ohtani et al., 1999); (b) norepinephrine concentration increased during heat stress (Myers and Chinn, 1973); and (c) both the neurotransmitters are involved in thermoregulation (Mallick et al., 1992; Clark and Lipton, 1985). Since isolated studies have shown that NE and GABA are affected by changes in body temperature, it is reasonable to argue that both the neurotransmitters are likely to interact and modulate the neuronal activity of at least the thermosensitive neurons in mPOAH. This may be supported by the fact that the interaction between NE and ACh in mPOAH for the regulation of S-W and thermoregulation in rats have been reported from this lab (Mallick and Joseph, 1998). Based on the results of this study a model is being proposed (Fig. 5.1).

It has been observed that in the mPOAH, both the WSNs and CSNs receive excitatory noradrenergic inputs but GABA exerted excitatory and inhibitory inputs on these neurons, respectively. On the other hand, it has been reported that GABA evoked norepinephrine release by activating GABA-A receptors and GABA transporters (Fassio et al., 1999). Therefore, it can be said that GABA in the mPOAH may have a direct inhibitory action on the CSNs while it has an indirect excitatory action on the WSNs, which may be mediated by NE, for the regulation
of body temperature. GABA possibly acts on the GABA-ergic heteroreceptors on the noradrenergic presynaptic terminals for the release of NE. The possible mechanism of GABA-A mediated induced release of NE may be supported by the fact that the GABA-A receptors evoked NE release by conventional exocytosis (Fillenz, 1990). It has further been shown that the NE release elicited through GABA-A receptors was Ca^{2+}-dependent (Fassio et al., 1999); only N-type Ca^{2+}-channels and not P-type or Q-type Ca^{2+}-channels are involved in the process.

Our model may be supported by the report that the concentration of both norepinephrine and GABA decreased after exposure of rats to cold (Oltani et al., 1999) and that norepinephrine concentration increased during heat stress (Myers and Chinn, 1973). GABA and norepinephrine and their agonists, both evoked hypothermia (Poole and Stephenson, 1979; Clark and Lipton, 1985; Mallick and Alam, 1992) while their antagonists evoked hyperthermia (Serrano et al., 1985; Mallick and Alam, 1992). Thus, when there is increased concentration of GABA it directly deactivates the CSNs but activates the WSNs through norepinephrine release causing stimulation of hypothermic responses and the body temperature tends to decrease. On the other hand, during reduced release of GABA, an opposite response is triggered which stimulates hyperthermia responses. Thus, it is hypothesized that GABA and NE interact in mPOAH to switch-on or switch-off the heat dissipating and heat generating mechanisms, as the case may be, for thermoregulation.

Brainstem stimulation excited WSNs and inhibited CSNs in the mPOAH. The effects of electrical stimulation of brainstem provided information about the physiological connection between the neurons located in and around stimulation site in the brainstem and the thermosensitive neurons recorded in the mPOAH. The NE-ergic and GABA-ergic inputs on the thermosensitive neurons could also be part of brainstem projections on thermosensitive neurons. Nevertheless, the response of thermosensitive neurons to brainstem stimulation could be direct or indirect.
Fig. 4.1 A diagrammatic representation of model showing a possible mechanism of interaction between GABA-ergic and NE-ergic inputs on the thermosensitive neurons in the mPOAH. NE through α-1 adrenoreceptors caused excitation of both types of thermosensitive neurons, whereas GABA inhibited CSNs directly and excited WSNs indirectly. Possibly GABA acted through GABA-A heteroreceptor at NE terminals and caused NE release which in turn caused excitation of WSNs. GABA-A receptors induced NE release by opening only N-type calcium channels while P and Q type calcium channels remain unaffected.
Physiological Significance

Based on the results of this study the proposed model helps in understanding the physiological significance and simultaneous regulation of sleep-wakefulness and body temperature at the cellular level. GABA is a dominant neurotransmitter in the mPOAH which induces sleep and hypothermia. Body temperature decreases during sleep and increases during wakefulness. Prolonged wakefulness results in sustained hyperthermia and increased concentration of GABA as well as of NE in the mPOAH. As per the proposed model, an increased turn over of GABA would also increase NE release. This increased NE would excite WSNs and GABA would inhibit CSNs resulting in hypothermia. The NE also excited CSNs, possibly at lower concentration, during sleep and hypothermia. It may have functional significance that depending on its concentration NE may either induce hyperthermic responses or would prevent excessive fall of body temperature during hypothermic state. Also the increased concentration of GABA during hyperthermia would possibly mask the excitatory effect of NE on CSNs.

Both types of neurons, sleep-wake related as well as thermosensitive neurons, have been reported to be present in the mPOAH and brainstem. Brainstem stimulation as well as NE both induces wakefulness and thermoregulatory responses. The thermosensitive neurons are also involved in the modulation of sleep-wakefulness. The brainstem stimulation induced primary excitation and inhibition of WSNs and CSNs signify that neuronal circuitry of brainstem to the mPOAH may be involved in the simultaneous modulation of sleep-wakefulness and thermoregulation. Hence, it can be said that the brainstem while regulating sleep-wakefulness, also keeps a control over the thermosensitive neurons to maintain body temperature within the physiological limits. The GABA-ergic interneurons and NE-ergic inputs probably arising from the brainstem on the thermosensitive neurons in the mPOAH possibly would prevent the subjects to go into excessive hyperthermic or hypothermic condition in case of prolonged wakefulness or sleep, respectively.
Based on the results following conclusion can be drawn:

1. Picrotoxin microinjection into the mPOAH produced wakefulness and hyperthermia. Hence, GABA acting on GABA-A receptors in the mPOAH is involved in spontaneous and simultaneous modulation of S-W and body temperature.

2. The Pearson correlation test showed that brain and body temperatures are positively correlated. The brain temperature had a tendency to increase along with the body temperature but it varied within a relatively narrower range as compared to that of the rectal temperature. Thus, brain has a better mechanism of heat regulation so that it is not unnecessarily stressed by heat or cold. This is significant considering the sensitive nature of the brain.

3. Microiontophoretic application of picrotoxin, a GABA-A receptor blocker, showed that GABA-A heteroreceptors possibly regulate the NE release, which along with GABA modulate neuronal activity of thermosensitive neurons in the mPOAH.

4. The results of microiontophoretic injection of prazosin, an α-1 adrenoreceptor blocker, showed the presence of α-1 adrenoreceptors on thermosensitive neurons. It substantiated the earlier hypothesis put forward from this laboratory. The results also indicated that GABA and NE interact at the synaptic level for regulation of the activity of thermosensitive neurons.

5. The brainstem projects to the thermosensitive neurons in the mPOAH. The excitation and inhibition of WSNs and CSNs by brainstem stimulation showed that brainstem, a wakefulness inducing area, also modulates the thermosensitive neuronal activity. Such modulation possibly plays a significant role in thermoregulation within physiological limits during sleep-wakefulness.