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# PREOPTIC AREA SLEEP-REGULATING MECHANISMS

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## INTRODUCTION

In the second edition of *Sleep and Wakefulness* (21), in a chapter he called "The Sleep-Center Problem", Kleitman considered the evidence for and against the existence of a localized sleep-promoting neuronal system in the brain. With characteristic thoroughness, he first summarized the theoretical localization schemes proposed for such a sleep center. As a starting point, Von Economo's (64) conceptualization of dual, mutually antagonistic hypothalamic sleep- and waking-centers was considered at some length. Kleitman then reviewed a literature that variously proposed to localize the sleep center to peraqueductal, periventricular, thalamic or neocortical sites, on the basis of clinical findings and/or theoretical speculation.

He then turned his critical eye to what he referred to as "the factual side", i.e., the experimental evidence derived from stimulation and lesion studies in animals. He was not impressed with the ability of diencephalic stimulation to induce sleep in the cat as described by Hess (15), because of the finding that "one can awaken a cat, put to sleep by electrical stimulation, by applying a higher voltage to the same spot". He dismissed the findings of Nauta (35) that rostral midline hypothalamic damage induces complete sleeplessness, because the animals did not live for more than a day. Noting that one guinea pig with a similar lesion described by Manceau and Jordan (28) survived for >3 weeks , Kleitman wryly concluded that "The proof of the presence of an anatomically separate sleep center at present rests on that one guinea pig's induced 26-day sleeplessness".

Kleitman's own conclusion from the available evidence was unequivocal, "If there is a center, it is a waking one, concerned with the maintenance of waking activity". His belief in the concept of sleep as a passive process was in agreement with much of the theoretical and experimental evidence accumulated up to that time, and his critical evaluation of the existing literature in support of an active sleep promoting mechanism failed to convince him otherwise.

Our goal is to review some of the evidence accumulated since 1963 in support of a sleep-promoting neuronal mechanism localizable to the preoptic region of the hypothalamus. This will include the results of lesion, electrophysiological and neuroanatomical/neurochemical studies. An emphasis will be on recent progress that has been made in more precisely identifying and localizing putative sleep-promoting neuronal types, and defining the nature of their neuroanatomical and

functional interactions with arousal/activating neuronal systems located in the hypothalamus and brainstem. While our conclusions about the existence of a localizable sleep-promoting mechanism are at odds with Kleitman's, as is the case throughout Sleep and Wakefulness, his thoughtful consideration of the "Sleep Center Problem" defined the critical questions and set standards for the kinds of experimental evidence required to answer them.

## EVIDENCE FOR A SLEEP-PROMOTING FUNCTION IN THE LATERAL PREOPTIC AREA AND BASAL FOREBRAIN OF CATS

Roughly coinciding with the publication of the second edition of *Sleep and Wakefulness*, Sterman and Clemente (55) first described the ability of electrical stimulation in selected regions of the basal forebrain to induce sleep with cortical EEG synchronization in the cat. The most effective sites were located in the preoptic area (POA), including the horizontal limb of the diagonal bands (hDBB) rostral to the optic chiasm and in the ventral-most portion of the lateral POA, both just rostral to the optic chiasm and at the level of the chiasm. In contrast to stimulation effects described by Hess, both low and high frequency stimulation of these basal forebrain sites promoted sleep (6, 55).

Bilateral electrolytic lesions involving the ventral lateral preoptic area (vlPOA) and/or the hDBB in cats resulted in significant reductions in both nonREM and REM sleep (27). Sleep disruption persisted for 3 weeks. Peak levels of insomnia were found during the first postlesion week, with total sleep time declining by >50% compared to baseline. Similar effects on sleep were observed following axon-sparing excitotoxin-induced basal forebrain lesions in cats (59). While centered in the vlPOA and hDBB, the area of cell loss also extended dorsally and laterally. Following such lesions, amounts of deep nonREM sleep were suppressed for as long as 6 weeks postlesion, compared to baseline. Peak sleep disturbance was observed during postlesion weeks 1 and 2, with >50% reduction in total sleep time as a result of significant decrements in both nonREM and REM sleep.

Extracellular recording of single neuronal activity in these regions of the cat basal forebrain revealed populations of neurons with nonREM sleep-related discharge patterns, i.e., discharge rates generally <1 Hz in waking, increasing discharge rates during transitions from waking to sleep and peak discharge rates during deep, stable nonREM sleep (58, 61). In all basal forebrain regions examined, these "sleep-active" cell types were intermixed with neurons that were active during waking or neurons that did not show strong modulation of discharge rates across the sleep-waking cycle. In fact, waking-active neurons were the most prevalent cell type in all regions studied, suggesting that the sleep-promoting neuronal system might be diffusely organized in this species and not anatomically segregated from other cell types.

## EVIDENCE FOR A SLEEP-PROMOTING MECHANISM IN THE MEDIAL PREOPTIC AREA AND ANTERIOR HYPOTHALAMUS

In the lesion studies reviewed above, persistent insomnia was induced as a result of damage centered in the lateral portions of the POA, with substantial or complete sparing of the medial POA (mPOA) and anterior hypothalamus adjacent to the third ventricle. The transection lesions of Nauta (35) that resulted in insomnia shortly followed by death destroyed these midline regions. Subsequently, large, bilateral electrolytic lesions centered in the mPOA were shown to produce severe and persistent sleep disturbance in cats (32). Two animals exhibited completed sleeplessness for periods ranging from 2-7 days, followed by death on postlesion day 10. Surviving cats displayed significant reductions in all sleep stages, that reached maximum severity during the first and second postlesion weeks. Significant sleep disturbance persisted for one month postlesion. The importance of destruction of mPOA neurons in mediating these effects, as opposed to axons passing through the POA region, was confirmed by the ability of axon-sparing excitotoxin-induced lesions of the mPOA to cause severe and persistent insomnia in cats (46, 47, 57).

Similar results have been described following mPOA lesions in rats. Large, bilatleral electrolytic lesions that extended to portions of the anterior hypothalamus caudally and to midline tissue just rostral to the lamina terminalis, caused suppression of both nonREM and REM sleep in rats (62). Near complete insomnia was observed during the first 2 days postlesion (total sleep time reduced by 85% compared to baseline), and sleep disturbance remained severe over the next 4 weeks (total sleep time reduced by an average of 60% compared to baseline). Excitotoxin-induced mPOA lesions of similar rostral-caudal extent, resulted in >50% reductions in total sleep time in rats (16). Sleep disturbance did not exhibit significant improvement during the 22 day observation period. The insomnia induced by these excitotoxin lesions was partially reversed following transplantation of fetal preoptic a tissue into the damaged preoptic area (17).

Putative sleep-promoting neurons have been identified in the preoptic area of several species on the basis of extracellular recordings during natural wakefulness and sleep. Neurons with a nonREM sleep-related discharge pattern have been identified throughout the preoptic/anterior hypothalamic area in rabbits (10), cats (1, 20), and rats (2, 22, 23, 39). Sleep-active neuronal types were not obviously segregated from neurons with waking-related discharge patterns, again suggesting a diffuse organization of sleep-promoting neuronal types.

In addition to a role in sleep, the mPOA has long been implicated in thermoregulatory function, and is a major thermosensing and thermointegrating region of the mammalian brain (48). Thermoregulatory and sleep-regulatory neuronal systems of the preoptic area appear to be anatomically and functionally related. In nearly every case, preoptic area damage that was effective in causing insomnia, also caused abnormalities in body temperature (16, 32, 46, 57, 62). In both cats and rats, manipulation of environmental temperature with respect to the thermoneutral zone,

alters the severity of both nonREM sleep and REM sleep disturbances (57, 62). Animals with mPOA lesions exhibit reduced threshold for sleep disruption when the ambient temperature is changed to either above or below thermoneutral values. At an optimum ambient temperature sleep disturbances can be significantly improved, at least acutely. Increased sensitivity of sleep measures to environmental temperature changes can occur in preoptic-damaged animals even when there is no overt abnormality in spontaneous body temperature levels (62). Therefore, claims that the sleep and thermoregulatory deficits of preoptic area damage can be dissociated (16, 46) should be interpreted with caution when measures of resting body temperature constitute the only assessment of thermoregulatory function.

Evidence for a functional interaction between preoptic sleep- and thermosensitive mechanisms is provided by unit recording and thermal stimulation studies. In both cats and rats, neuronal sleep-waking discharge patterns are correlated with local thermosensitivity of neuronal discharge (1, 2). A majority of POA warm-sensitive neurons (ie., those that exhibit an increase in discharge rate in response to local increases in temperature) also display sleep-related neuronal discharge patterns. A majority of cold-sensitive neurons display waking-related discharge patterns. If these thermosensitive neurons have a functional role in sleep-regulation, one would predict that local manipulations of preoptic area temperature should alter sleep-wake state amounts. For example, local preoptic area warming should activate a population of sleep-related/warm-sensitive cell types and suppress activity of waking-related/cold sensitive neurons. In fact, focal POA warming has been demonstrated to augment nonREM sleep in several species (5, 42, 43, 45), and to increase cortical EEG synchrony in cats (30). Interactions between POA sleep- and thermoregulatory mechanisms is considered in more detail by McGinty et al elsewhere in this volume.

## IMPROVED LOCALIZATION OF PUTATIVE PREOPTIC AREA; SLEEP-REGULATORY NEURONS WITH THE *c-fos* IMMUNOHISTOCHEMICAL METHOD

As summarized above, the experimental findings that large preoptic lesions are required to produce severe and persistent sleep disturbance, and that neurons with sleep-related neuronal discharge are not anatomically segregated from other cell types, has led to the perception that POA sleep-promoting neuronal systems are diffusely organized. There is now a need to at least partially revise this view, based on findings of localized groups of POA neurons that exhibit increased c-fos gene expression during sleep.

*c-fos* is an immediate early gene whose expression has been found to correlate with increased neuronal activity in a variety of cell types. Sherin et al. (51) were the first to describe a discrete cluster of cells in the vlPOA that exhibit immunoreactivity for the fos protein during sleep (Figure 1C). The number of fos-immunoreactive (ir) cells was found to be positively correlated with the time spent asleep during the one-hour period prior to sacrifice. Fos-ir neurons in the vlPOA

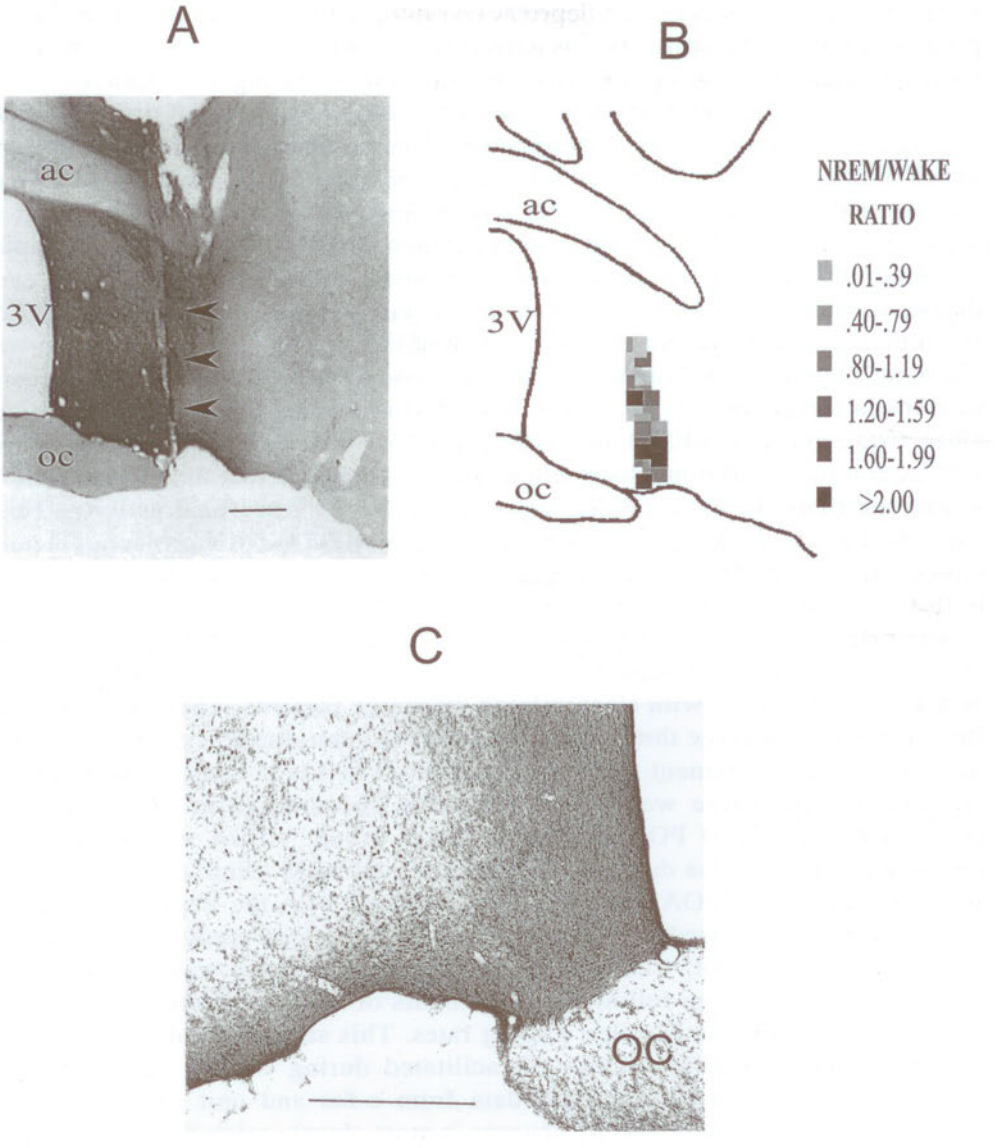


Fig. 1. - *Distribution of neurons with sleep-related neuronal discharge (A and B) and neurons exhibiting sleep-related fos-ir (C) in the lateral POA of rats.*

A. Photomicrograph of a microwire pass (indicated by arrows) through the lateral POA, extending from just ventral to the anterior commissure to the base of the brain. Calibration 500μ. B. Reconstruction of the microwire pass in A, with the locations of all recorded neurons indicated by squares. NonREM sleep to waking discharge rate ratio of each cell is shown by a gray scale, with black indicating neurons with the most strongly sleep-related discharge profiles. Note the concentration of sleep-related neurons at the most ventral portions of the microwire pass. C. Photomicrograph showing distribution of fos-ir neurons in the lateral POA from a rat that spent >70% of the time asleep during the hour prior to sacrifice. Calibration 500 μ. A and B modified from reference; C unpublished observations. Abbreviations: ac, anterior commissure; oc, optic chiasm; 3V, third ventricle.

were observed following sleep deprivation only if animals were permitted to sleep prior to sacrifice. Therefore, c-fos activation appeared to be related to the occurrence of sleep, not sleep propensity, or "sleepiness" during wakefulness.

Fos-ir neurons in the ventrolateral preoptic area were found to project to histaminergic cell groups of the tuberomammillary nucleus (TMN) of the posterior hypothalamus (50, 51). TMN neurons are the only source of histamine in the brain, and the TMN histaminergic system has widespread projections throughout the brainstem, diencephalon, limbic system and neocortex (65). Brain histamine plays an important role in the regulation of arousal. Histaminergic agonists evoke depolarizations in thalamocortical neurons and promote wakefulness (25, 29). Histaminergic antagonists promote sleep and EEG synchrony (25). The sleep-wake discharge pattern of TMN neurons resemble those of serotonergic and noradrenergic neurons in the brainstem; i.e., tonic firing during waking and significantly diminished firing during nonREM and REM sleep (52, 63). The dense projection from vlPOA neurons exhibiting sleep-related fos-ir, suggested that inhibition of vlPOA might contribute to sleep-related suppression of TMN neuronal activity. This is supported by the finding that a large majority of vlPOA-TMN projection neurons contain the inhibitory neurotransmitter gamma-amino-butryic acid (GABA), or both GABA and the inhibitory neuropeptide gallanin (11, 50).

The finding of a highly localized group of cells in the vlPOA that exhibit fos-ir as a consequence of sleep, suggests that this region should contain a high concentration of cells with sleep-related discharge patterns. Therefore, we examined neuronal discharge throughout the dorsal to ventral extent of the lateral POA of rats (56). In agreement with the distribution of fos-ir neurons, neurons with sleep-related discharge were most frequently encountered in the ventral most portions of the lateral POA (Figure 1). As a group, vlPOA neurons displayed elevated discharge rates during both nonREM and REM sleep, compared to waking. Discharge of vlPOA neurons was correlated with the depth of sleep, i.e., discharge rates increased significantly from light to deep nonREM sleep (Figure 2). We also found that, while discharge of vlPOA neurons was elevated during recovery nonREM sleep following 12-14 hours of sleep deprivation, rates during waking were similar to baseline waking rates. This suggests that vlPOA neurons can be strongly inhibited and/or disfacilitated during waking after significant periods of sleep deprivation. Thus data from c-fos and unit recording studies indicate that activation of vlPOA neurons is most closely related to the occurrence and depth of sleep, but not to sleep propensity during wakefulness (51, 56).

As mentioned previously, discharge of vlPOA neurons during both nonREM and REM sleep were significantly higher than waking discharge rates, but nonREM and REM sleep rates did not differ significantly. This is the exact reciprocal pattern of state dependent discharge observed in monoamine cell groups, including putative histaminergic neurons of the TMN. This would be predicted from the existence of a strong GABAergic projection from the vlPOA to the TMN. A comparison of state-dependent discharge rates of neurons recorded in the vlPOA of rats and of putative histaminergic neurons in the rat TMN is shown in Figure 3.

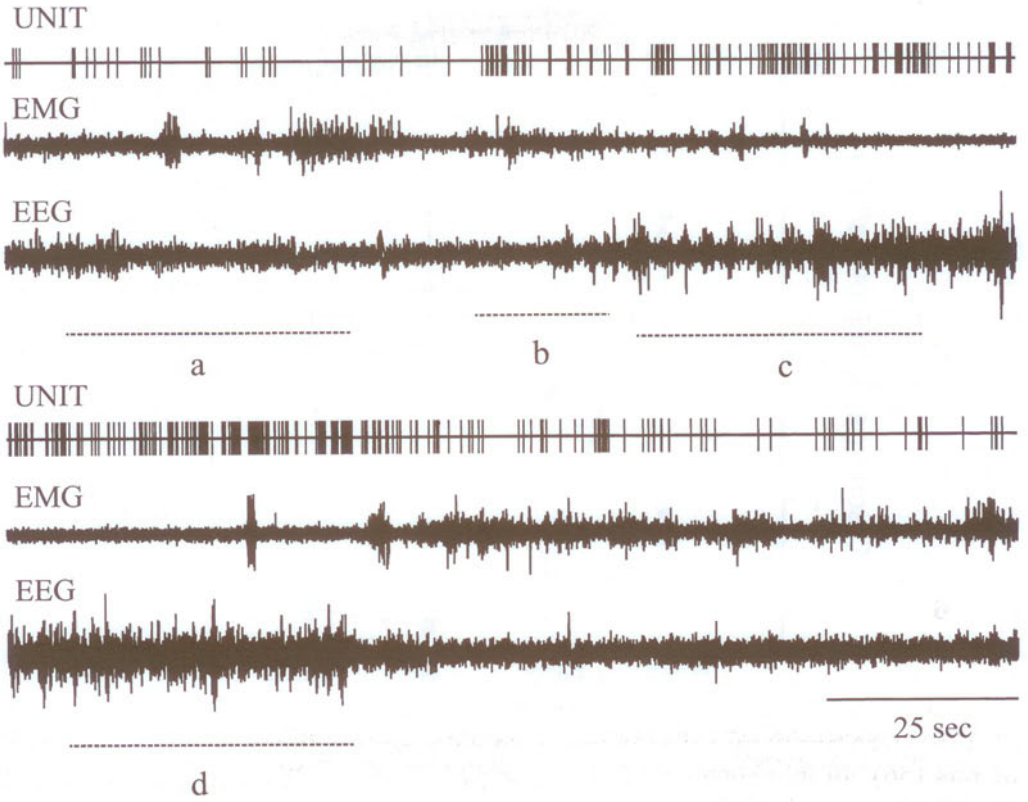


Fig. 2. - *Example of a vlPOA neuronal discharge during consolidated waking (dotted line 'a'), the wake to nonREM transition period (dotted line 'b'), early nonREM sleep (the initial one-third of the nonREM sleep episode; dotted line 'c'), and late nonREM sleep (dotted line 'd').*

From Ref. 56.

Recent anatomical studies indicate that vlPOA neurons may exert inhibitory modulatory control over brainstem monoamine cell groups. Placement of anterograde tracer injections into the vlPOA region, in addition to densely labeling the TMN region, yield labeled axons in the serotonergic dorsal raphe nucleus and in noradrenergic regions of the locus coeruleus (Figure 4) (50, 53). These finding, combined with what is known about the sleep-wake discharge profiles of vlPOA neurons (56), suggests the hypothesis that activation of these neurons contributes to suppression of serotonergic and noradrenergic neurons during nonREM sleep and REM sleep.

The discovery of such a highly localized group of putative sleep-regulatory neurons in the vlPOA, prompts a re-assessment of previous lesion and neuronal recording studies suggesting a critical role for the mPOA in sleep control. Is the insomnia-induced by large bilateral mPOA lesions primarily the result of damage to the adjacent vlPOA region? Are neurons with sleep-related discharge in mPOA

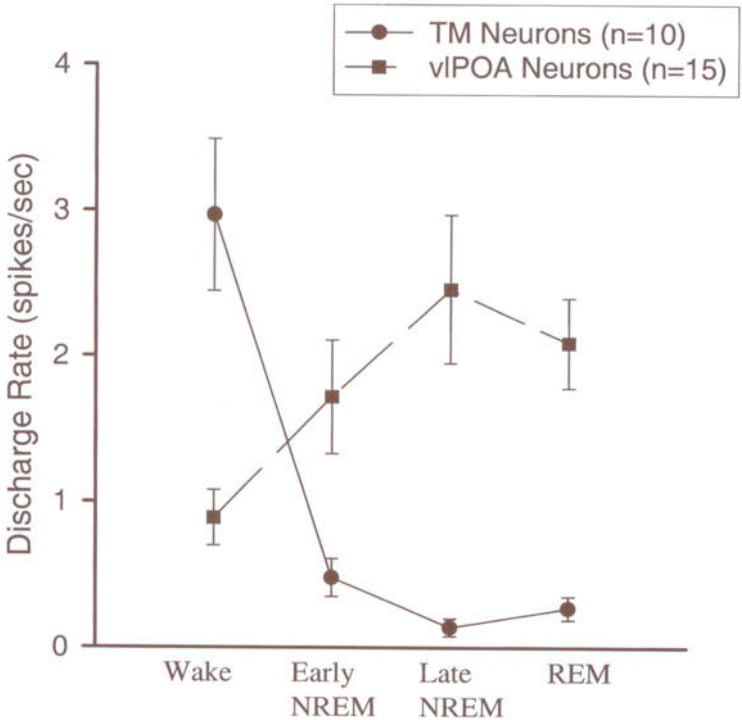


Fig. 3. - *Comparison of sleep/wake state-dependent discharge of neurons with sleep-related discharge in the vlPOA and putative histaminergic neurons exhibiting REM-off discharge patterns recorded in the tuberomammillary nucleus (TM).*

Discharge rates were calculated during waking, early nonREM sleep, late nonREM sleep and REM sleep. Note the reciprocal relationship of discharge patterns across the sleep-wake cycle between these two cell types. Data modified from Refs. 56 and 52.

so extensively intermixed with waking-active neuronal types such that they cannot be localized by comparing the distribution of fos-ir neurons as a consequence of waking and sleep?

A partial understanding of the role of the mPOA and vlPOA regions comes from recent studies demonstrating that neurons exhibiting fos-ir during sleep extend beyond the localized cluster of neurons originally identified in the vlPOA. Examination of fos-ir combined with in situ hybridization for gallanin, reveals that >80% of the fos-ir neurons in the vlPOA cluster contain gallanin, and that fos/gallanin positive neurons extend medially and dorsally from the vlPOA cluster (11). The medial extension includes regions extensively damaged in most mPOA lesion studies of sleep, and regions where neurons with sleep-related discharge have been described. Anterograde tracer injections placed in the regions of the medial and dorsal extensions yield labelled axons in the TMN, dorsal raphe nucleus and locus coeruleus, although the density of labeling is lower than that observed after vlPOA injections (51, 53) (Figure 4).

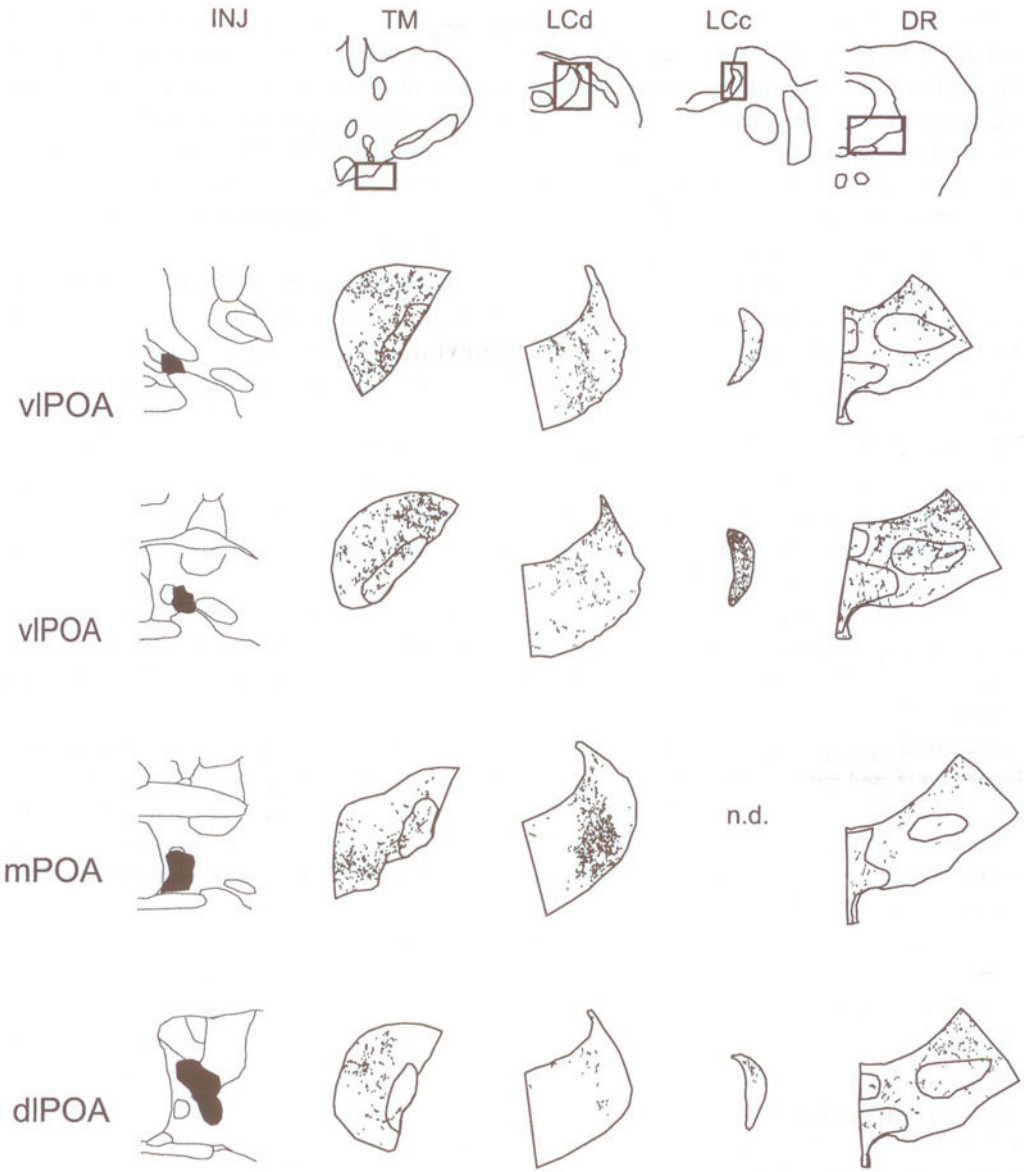


Fig. 4. - *Sites of anterograde tracer (biotinylated dextran; BD) injections into various POA subregions and corresponding projection targets in the tuberomammillary nucleus (TM), locus coeruleus (cell body region LCc; dendritic region LCd) and dorsal raphe nucleus (DR).*

Top panels show coronal sections at the levels of the TM, LC and DR. The areas indicated by the boxes are expanded below to demonstrate locations of BD labeled axons as a consequence of the BD injection placements shown in the left panels. Two of the injection sites shown were centered in the vlPOA cell cluster region, one in the mPOA encompassing the area of the medial vlPOA extension, and one placed dorsal to the vlPOA cell cluster region. n.d. indicates that distribution of BD labeled axons was not determined in the LCc region for the mPOA injection site. Data modified from Ref. 53.

We have recently found that neurons in the median preoptic nucleus (MnPN) exhibit fos-ir during sleep in rats (12). Dense, sleep-related fos-ir is observed throughout the rostral to caudal extent of this nucleus, and is enhanced in rats sleeping in a warm environmental temperature This suggests that both sleep and thermoregulatory factors can influence fos expression in MnPN neurons. Whether or not these neurons contain galanin and/or GABA remains to be determined, but these findings indicate a much wider distribution of neurons expressing sleep-related fos-ir than had been previously appreciated.

A recent lesion study has attempted to determine the role of the vlPOA cell cluster and the dorsal and medial extension of this cluster in sleep-regulation (26). Sleep-wake disturbance was examined following placement of lesions that were designed to destroy neurons in the vlPOA cluster and in the regions medial and dorsal to it. Lesions were made with the excitotoxin, ibotenic acid. Comparisons of damage to the vlPOA cluster and its extensions were made by quantifying the number of fos-ir neurons remaining in animals sacrificed between circadian times 3-5 (lights-on at circadian time 0). These are times when rats normally sleep >50% of the time, but no direct measures of sleep amounts were made in individual animals prior to sacrifice.

When cell damage was centered in the vlPOA cluster, destroying >80% of the fos-ir neurons, rats exhibited profound acute sleep loss (56% reduction in total sleep time; 54% reduction in nonREM sleep time, and 58% reduction in REM sleep time). The magnitude of the decrease in nonREM sleep time was significantly correlated with loss of fos-ir neurons in the vlPOA cluster, but the correlation between REM sleep loss and loss of fos-ir neurons was not significant. Rats with bilateral lesions in the mPOA that substantially spared fos-ir neurons in the vlPOA cluster, exhibited 25% reduction in nonREM sleep and 31% reduction in REM sleep. Sleep loss in the mPOA damaged rats was comparable to those observed in rats with vlPOA damage during the lights-on period. During the lights-off period reductions in the sleep of rats with mPOA damage was not as pronounced, in part, because these animals had less sleep at night during baseline recordings.

Following lesions centered in the region of the dorsal extension, an even larger discrepancy was found between the magnitude of the decrease in nonREM versus REM sleep in the acute postlesion period (15% versus 35%). These finding were interpreted as evidence that the integrety of the vlPOA cluster of neurons is critical for nonREM sleep regulation, and that the medial and dorsal extensions may be more importantly involved in REM sleep control.

Rats with lesions that extensively damaged the vlPOA cluster did not exhibit any persistent abnormalities in resting body temperature or in the circadian rhythm in body temperature, suggesting a dissociation of sleep and thermoregulatory disturbances. However, sleep-wake measures were performed only at a single ambient temperature that was outside the thermoneutral zone for the rat (i.e., 20-22(C). Therefore, the possibility remains that rats with neuronal loss that includes the vlPOA cell cluster display increased sensitivity of both nonREM and REM sleep measures to deviations from thermoneutrality.

No assessment of thermoregulatory function was made in rats with mPOA lesions that caused insomnia (26). Rats with small lesions confined to the ventromedial POA had no sleep disruption, but did show abnormalities in the circadian body temperature rhythm (26).

## DISCUSSION

Considerable progress has been made in characterizing the neurophysiology, neuroanatomy and neurochemistry of sleep promoting mechanisms in the POA. Putative sleep promoting neuronal types have been identified on the basis of lesion effects, neuronal discharge patterns, and sleep-related fos-ir (1, 12, 26, 51, 56). All three kinds of evidence point to the POA neurons as being critically involved in sleep regulation. Neurons in the vlPOA cluster, as well as neurons extending medially and dorsally, contain the inhibitory neurotransmitters/ neuromodulators GABA and gallanin (11, 50) and project to hypothalamic and brainstem regions implicated in arousal (13, 50, 53). These regions include the TMN and adjacent posterior hypothalamus, the dorsal raphe nucleus and the locus coeruleus. These finding combined with the sleep-wake discharge profiles of neurons recorded in the lateral and medial POA, support the hypothesis that POA neurons are a source of GABA-mediated inhibitory control of monoaminergic systems during both nonREM and REM sleep.

This hypothesis is further supported by several physiological findings. 1) Extracellular levels of GABA in the posterior hypothalamus, dorsal raphe and locus coeruleus are elevated during sleep compared to waking (36-38). 2) Cats with insomnia subsequent to excitotoxin-induced lesions of the POA exhibit restoration of sleep following microinjection of the GABA agonist, muscimol, into the posterior hypothalamus (47). 3) Electrical stimulation of the POA in an in vitro horizontal slice preparation of the rat brain that contains both the POA and TMN, evokes GABA-mediated inhibitory postsynaptic potentials in histaminergic neurons (54, 66). 4) Focal warming of the POA, a manipulation that activates neurons with sleep-related discharge in the mPOA and vlPOA (1, 56), causes suppression of spontaneous waking discharge in the posterior hypothalamus and dorsal raphe nucleus (14, 24). Thus, there is strong experimental support for the existence within the POA of a descending inhibitory system that promotes sleep, at least in part, via suppression of multiple activating systems in the hypothalamus and brainstem.

Important questions about POA sleep-regulating mechanisms remain to be answered. What factors are responsible for modulating the activity of POA sleep-promoting neuronal systems? Both fos-ir and unit recording studies suggest that activation of vlPOA neurons occurs late in the transition sequence from waking to sleep (51, 56). The activity of vlPOA neurons remains low during waking even after significant sleep deprivation (56). The mechanisms responsible for inhibiting POA sleep-related neurons during waking and/or causing disinhibition/excitation during sleep are unknown.

Mutual inhibitory interactions may exist between POA sleep-related neurons and the monoamine arousal systems. The reciprocal nature of the state-dependent discharge patterns of vlPOA and TMN neurons is suggestive of such an interaction (see Figure 3). POA sleep-related neurons display a rapid suppression of discharge at sudden transitions from waking to sleep (1, 2, 56), further suggesting that activation of arousal-related afferents might be an important source of inhibitory control.

Endogenous sleep factors may also be involved in modulating the activity of POA sleep-regulating neuronal systems. Activation of fos-ir in the vlPOA and MnPN accompanies sleep induced by infusion of prostaglandin D2 into the subarachnoid space just ventral to the POA (49). Adenosine analogs cause disinhibition of vlPOA neurons recorded in *vitro*, suggesting a mechanism by which increases in extracellular adenosine as a consequence of prior wakefulness could act to promote sleep (41).

What are the functional roles of the various populations of sleep-related neurons that have been identified in the POA? These include the GABAergic/gallanergic neurons of the vlPOA and its medial and dorsal extensions, the neurons of the MnPN that exhibit sleep-related fos-ir, and the neurons that are both sleep-related and warm-sensitive that have been recorded throughout the POA and the hDBB.

Neurons in the vlPOA and its extensions may have a special role in regulating the activity of multiple monoaminergic arousal systems, as suggested by anatomical and physiological studies, reviewed above. However, destruction of monoamine cell groups does not cause persistent arousal deficits (4, 19, 44), and fully activated neocortical EEG patterns persist during REM sleep when activity of histaminergic, serotonergic and noradrenergic neurons are at their minimum (4, 14, 31, 52, 63). Therefore additional brain arousal mechanisms must be critically involved in sleep-wake regulation.

Additional important sources of thalamocortical activation during waking and REM sleep include cholinergic neurons of the dorsolateral pons and magnocellular basal forebrain regions, and excitatory amino acid neurons of the pontine and midbrain reticular core extending to the level of the posterior lateral hypothalamus (see 18 for review). The recently described collection of neurons in the perifornical region of the hypothalamus that contain the neuropeptide, hypocretin (orexin), and that project to widespread cortical and subcortical sites, constitutes another potential important arousal system (7, 40).

The thermosensitive component of the POA sleep-regulatory mechanism has been implicated in modulating neurons in some of these nonmonoaminergic arousal systems. Focal warming of the POA evokes suppression of spontaneous and evoked waking neuronal discharge in putative cholinergic neurons in the magnocellular basal forebrain (3) and of arousal-related neurons in the posterior lateral hypothalamus (24). Both thermal (8) and electrical (60) stimulation of the POA evoke suppression of neuronal discharge in the midbrain reticular formation. The presence or absence of projections from the MnPN, hDBB and other POA regions implicated in sleep regulation to one or more of these nonmonoaminergic arousal systems remains to be determined, as does the neurotransmitter/neuromodulator pheno-

types of POA sleep-active neurons located outside the vlPOA cluster and its extensions.

## SUMMARY

Evidence is summarized for the existence of a sleep-regulating mechanism within the preoptic area of the hypothalamus, including the results of lesion, stimulation, and neuronal recording studies. Recent findings employing the c-fos protein immunohistochemical method, have localized putative sleep-regulatory neurons to the ventrolateral preoptic area (vlPOA) and the median preoptic nucleus (MnPn). Electrophysiological studies have confirmed the presence of neurons with sleep-related discharge in the vlPOA. Neurons in the vlPOA that exhibit c-fos protein immunoreactivity during sleep contain the inhibitory neuromodulators galanin and gamma-aminobutyric acid (GABA). These neurons also project to monoaminergic arousal systems, particularly the histaminergic cell groups in the posterior hypothalamus. POA neurons can be hypothesized to provide sleep-related inhibitory control over multiple arousal systems in the forebrain and brainstem.

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